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THE RELATIVE EFFECTS OF THE RENAL PRESSOR AND THE SYMPATHETIC NERVOUS SYSTEMS ON VASCULAR CAPACITY

by

Donald Walter DuCharme

A dissertation submitted in partial fulfillment
of the requirements for the degree of
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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	vii
CHAPTER	
I. INTRODUCTION	1
A. Historical review	1
1. The sympathetic nervous system	1
a. Control of arterial tone	2
b. Control of venous tone	4
2. The renal pressor system	7
a. Discovery and development	7
b. Site of renin formation and factors controlling secretion	11
c. Effect of angiotensin on the vasculature	17
B. Statement of the problem	22
II. MATERIALS AND METHODS	23
A. Controlled hypotension	23
B. Surgical procedures	28
1. Nephrectomy	28
2. Sham-operated	28
3. Tracheotomy and exposure of femoral artery	28
C. Spinal anesthesia	29
D. Perfusion of the hindquarters	29

CHAPTER Page

III. RESULTS 34

A. Bleeding volumes of sham-operated and bilaterally nephrectomized dogs subjected to severe hypotension 34

1. Control animals 34

2. Animals with spinal anesthesia . . . 34

3. Animals pretreated with phenoxybenzamine 43

4. Animals pretreated with guanethidine 47

B. Treatment interactions 51

C. Effect of various drug treatments on pre-hemorrhage blood pressures 54

D. Effect of various procedures and agents on the vasoconstrictor action of angiotensin in the perfused hindlimb of the dog 54

1. Interruption of adrenergic tone . . . 58

2. The ability of pre and postganglionic sympathetic nerve stimulation to restore the vasoconstrictor action of angiotensin following acute decentralization 61

3. Effect of spinal anesthesia 65

4. Effect of JB-516, guanethidine, reserpine, and Su-4029 65

5. Effect of cocaine 70

IV. DISCUSSION 73

A. The importance of the renal pressor system as a compensatory mechanism in acute hypotension 73

B. The effect of the sympathetic nervous system on vascular capacity in acute hypotension 81

C. The contribution of the heart to the bleeding volume 85

CHAPTER Page

D. The existence of an interaction between the sympathetic nervous system and the renal pressor system 88

E. Possible sites and mechanisms for the interaction between the sympathetic nervous system and the renal pressor system 92

SUMMARY 105

BIBLIOGRAPHY 107

LIST OF TABLES

TABLE		Page
1.	Effect of nephrectomy on bleeding volumes of control animals	37
2.	Effect of nephrectomy on secondary bleeding volumes of control animals.	38
3.	Effect of nephrectomy on bleeding volumes of animals with spinal anesthesia	40
4.	Effect of nephrectomy on secondary bleeding volumes of animals with spinal anesthesia	44
5.	Effect of nephrectomy on secondary bleeding volumes of animals pretreated with phenoxybenzamine.	48
6.	Bleeding volumes of guanethidine treated animals compared to those of control animals.	52
7.	Effect of spinal anesthesia on vascular capacity of guanethidine pretreated and normal dogs.	53
8.	Effect of phentolamine on vascular capacity of guanethidine pretreated and normal dogs.	55
9.	Effect of spinal anesthesia on vascular capacity of guanethidine pretreated and normal dogs previously treated with phentolamine	56
10.	Effect of drug treatments on pre-hemorrhage blood pressures	57
11.	Effect of stimulation frequency on restoration of angiotensin response	66
12.	Effect of pharmacologic agents on potentiation of the angiotensin response by lumbar sympathetic nerve stimulation	68

LIST OF FIGURES

FIGURE		Page
1.	Schematic diagram of the system utilized for blood pressure stabilization	25
2.	Schematic diagram of the perfusion system utilized for autocirculation of the hind limbs .	31
3.	Effect of nephrectomy on bleeding volumes of control animals and animals with spinal anesthesia	36
4.	Effect of nephrectomy on secondary bleeding volumes of control animals and animals with spinal anesthesia.	42
5.	Effect of nephrectomy on bleeding volumes of animals pretreated with phenoxybenzamine	46
6.	Effect of nephrectomy on bleeding volumes of animals pretreated with guanethidine	50
7.	Effect of neurogenic activity on the vasoconstrictor action of angiotensin.	60
8.	Correlation of neurogenic tone in the perfused extremities and the vasoconstrictor action of angiotensin.	63
9.	Effect of cocaine on restoration of the angiotensin response by sympathetic nerve stimulation.	72
10.	Schematic illustration of the contribution of the various systems to the bleeding volume . . .	103

CHAPTER I

INTRODUCTION

A. Historical review

It has long been accepted that a decrease in arterial pressure reflexly activates the sympathetic nervous system, which in turn causes generalized arteriolar constriction. Thus the sympathetic nervous system functions as a homeostatic mechanism for the regulation of systemic blood pressure. Sapirstein et al. (1941) demonstrated that upon hemorrhaging an animal a pressor substance of renal origin could be detected in the blood. This observation led them to postulate that the renin-angiotensin system is also a homeostatic mechanism for the maintenance of systemic blood pressure. Although many investigators have confirmed the fact that the renin-angiotensin system is indeed activated following hemorrhage, its physiological contribution as a compensatory system has never been quantitatively assessed. The present investigation was undertaken in an attempt to compare the effects of the sympathetic nervous system and the renal pressor system in the control of vascular capacity. The remainder of this chapter will be devoted to a review of the literature pertinent to this subject.

1. The sympathetic nervous system.

In view of the voluminous literature available concerning the sympathetic control of the cardiovascular system, only a brief survey of the more significant advancements will be presented. For a more extensive coverage of this literature the reader is referred to the following reviews; McDowall (1935), Barcroft and Swan (1953), Folkow (1955), McDowall (1956),

Heymans and Neil (1958).

a) Control of arterial tone.

The concept that the sympathetic nervous system exerts an effect upon the tone of the arterial circulation had its inception with the work of Claude Bernard in 1851. Bernard demonstrated that following section of the cervical sympathetic nerves a vasodilatation occurred in the ear of the rabbit. The following year (1852), Brown-Sequard and Bernard working independently, both demonstrated that electrical stimulation of the peripheral end of the cut cervical sympathetic nerve led to contraction of the ear vessels. Thus it was established that the sympathetic nerves were able to exert an influence upon the contractile elements of the arterial vasculature, and also that at least some portions of the arterial circulation were continually under tonic neurogenic influence.

Cyon and Ludwig (1866) are credited as being the first to demonstrate that the tonic influence of the sympathetic nerves may be reflexly regulated. They found that stimulation of the central end of the depressor nerve in the neck of the rabbit resulted in marked bradycardia and systemic hypotension. They believed that the sensory endings responsible for initiation of this reflex were located within the heart, and were stimulated by changes in intracardiac pressure. These observations were subsequently confirmed and extended to include other species of animals by several groups of investigators (See Heymans and Neil, 1958).

The belief that the sensory receptors responsible for the depressor reflex were situated in the heart persisted until

1902, when Koster and Tschermak by the use of degeneration techniques, proved them to be located mainly in the arch of the aorta. They further demonstrated that the cell bodies of these fibers were located in the vagal ganglion.

It is interesting that actually the first report of reflex control of the cardiovascular system was made by Sir Astley Cooper (1836). Cooper demonstrated that cardioacceleration occurred upon occlusion of the common carotid arteries. However, he attributed the cardioacceleration to cerebral ischemia rather than to a reflex phenomenon. Several other investigators confirmed the observation of Cooper and similarly interpreted the result. The belief that cerebral ischemia could induce tachycardia and elevated blood pressure persisted in spite of the fact that Bayliss (1893) stated that cerebral blood flow was contributed primarily by the vertebral arteries, and therefore, occlusion of the common carotid arteries could not possibly produce marked cerebral ischemia. In addition, Siciliano (1900) demonstrated that neither occlusion of the vertebral arteries nor occlusion of the external and internal carotid arteries evoked cardiac acceleration or increased systemic blood pressure. Thus he too felt that the response to occlusion of the common carotid arteries could not be attributed to cerebral ischemia.

It was not until 1923, however, when Hering performed his classical experiments demonstrating that the carotid occlusion response was dependent upon nerve endings located in the region of the carotid sinus, that the concept of Cooper was finally refuted. Hering (1924) then went on to establish the role of

the carotid sinus in maintaining arterial pressure. Thus it was established that the carotid sinuses act as pressure receptors, and that as arterial pressure is increased the sinus receptors are excited and exert an inhibitory influence upon the medullary vasomotor center, thereby bringing about decreased activity in the sympathetic nerves. Conversely, a decrease in arterial pressure diminishes activity of the sinus receptors and allows sympathetic activity to increase.

b) Control of venous tone.

The information available regarding control of the venous tone by the sympathetic nervous system is far less extensive than that for the arterial circulation owing largely to the lack of adequate techniques to evalute responses of the veins. However, it has been unequivocably established that the veins are indeed influenced by the sympathetic nervous system.

The first report indicating that the veins are under neurogenic control was probably that of Goltz (1863), who demonstrated that following mechanical stimulation of the intestines, the content of the intestinal veins was increased by some 16 times the normal volume. Furthermore, if the spinal cord was left intact the content of the veins would gradually return to normal values. In 1864, Goltz demonstrated that venomotor tone was dependent upon nervous centers in the spinal cord and medulla, and therefore inferred that the efferent supply was via the sympathetic nerves.

Hooker (1918) utilizing a preparation of the dog's sigmoid colon, was the first to demonstrate directly that venous tone in the mammal could be influenced by neurogenic stimulation. He

showed that venous pressure in the isolated segment of intestine could be elevated by direct stimulation of the nerves supplying the vasculature of the segment, by stimulation of sensory nerves so as to evoke a central reflex increase of sympathetic activity, or by subjecting the animal to asphyxia.

Donegan (1921) confirmed the observations made by Hooker on the intestinal veins. He noted that the superficial leg veins were under tonic influence of the central nervous system as were the intestinal veins, but he was unable to show that superficial veins of the leg participated in reflex cardiovascular adjustments. His explanation for this inconsistency was that the superficial veins are concerned with temperature regulation and therefore, would likely be controlled by central areas other than those involved in the reflex responses.

Heymans et al. (1930, 1931) using perfused, innervated segments of mesenteric veins, successfully demonstrated that reflex activation of the sympathetic nervous system following occlusion of the common carotid arteries leads to both venoconstriction and arteriolar constriction, and conversely, when the carotid sinus pressure is elevated both venodilatation and arteriolar dilatation ensues. These observations have subsequently been confirmed by Fleisch (1931), who utilized perfused, innervated segments of the colic vein, and by Alexander (1954) who utilized splanchnic veins. Thus it would appear to be generally accepted that at least the intestinal veins are to a degree under the control of baroreceptor reflexes.

Shadle et al. (1958) appear to have been the first to report that venoconstriction can be demonstrated to occur in a

musculocutaneous bed upon electrical excitation of the nerves supplying the bed. They utilized weight changes of an isolated, perfused dog hindlimb as an index of changes in vascular capacity. Unfortunately these investigators were unable to examine reflex effects on their preparation.

An extensive study of the effects of sympathetic stimulation on the resistance vessels, capacity vessels, and fluid exchange functions of the hindquarters of the cat has been reported by Mellander (1960). He found that stimulation of the sympathetic nerves to the hindlimb at a frequency of 6 cps evoked a maximal effect upon the capacity vessels, whereas a stimulation frequency of 16 cps was necessary to attain a maximum response of the resistance vessels. With maximum contraction of the capacity vessels approximately one-third of the regional blood volume was expelled from the limbs. However, occasionally up to 40-50 per cent of the total blood volume in the hindquarters could be expelled by sympathetic stimulation.

Folkow et al. (1964) has reported that intestinal veins of the cat respond comparably to leg veins when the corresponding sympathetic nerves are electrically stimulated. A maximal response occurred at a stimulation frequency of 6 cps, during which 30-40 per cent of the regional blood volume was expelled.

It is especially important to note that Lewis and Mellander (1962) have reported that when the blood flow to the hindquarters of the cat was mechanically decreased, there was a progressive decline in both the resistance and the capacitance effects evoked by stimulation of the decentralized sympathetic nerves. They further noted that if the decreased flow was

maintained for a long enough period both responses could be abolished, and that upon restoration of normal flow both responses rapidly returned to normal. The response of the resistance vessels was found to be much more sensitive to the decreased blood flow than was the response of the capacity vessels. The authors explain this observation on the basis that the decreased sensitivity is due to the local accumulation of vasodilator metabolites, and that the resistance vessels are more sensitive to the effects of these metabolites than are the capacitance vessels. Similar observations have been made by Mellander and Lewis (1963), and by Lundgren et al. (1964) following hemorrhagic hypotension. Based upon the assumption that the differential loss of vascular responsiveness was in fact due to the accumulation of vasodilator metabolites, Mellander and Lewis interpreted their experiments to mean that the pre-capillary functions were more under the control of local metabolic factors than the extrinsic nerve supply, and that the post-capillary vessels were influenced to a greater extent by the extrinsic nerves than by local metabolites.

2. The renal pressor system.

a) Discovery and development.

The development of the renopressor system began in 1898 when Tigerstedt and Bergman demonstrated that crude saline extracts of kidneys elicited a pressor response when injected into anesthetized rabbits. They introduced the name "renin" for the vasopressor constituent of the extract, and believed it to be a direct acting substance. There was then a hiatus of over thirty years before further interest was dis-

played in this fundamental observation. In 1934, Goldblatt demonstrated that partial occlusion of the renal artery led to a stable form of experimental hypertension in dogs, and thus proposed the hypothesis that the kidney contained a material which when secreted leads to an elevated arterial blood pressure. This idea led to an all-out effort by many investigators to explain the mechanism involved in the production of hypertension due to partial occlusion of the renal artery. Subsequently, it was demonstrated by several groups that following a decrease in renal blood flow a substance capable of producing a pressor response was released from the ischemic kidney into the systemic circulation (See reviews by Goldblatt 1947, and Braun-Menendez 1956).

Kohlstaedt, et al. (1938) observed that when renin was perfused with Ringer's solution through the vessels of the dog's tail or rabbit's ear a pressor response of lesser magnitude was evoked than if perfused with plasma. They also noted that as the renin was more highly purified the pressor response became less, but could be restored by the addition of plasma. This observation led them to propose that renin was acting as an enzyme, and the substrate "renin activator" was contained in the blood.

Braun-Menendez, et al. (1940), and Page and Helmer (1940) working independently and using very different approaches, both succeeded at about the same time in isolating the pressor material released from the kidney. Unfortunately, each group named the substance differently, and a great deal of confusion resulted as a consequence. Braun-Menendez et al. called the

substance "hypertensin," while Page and Helmer named it "angiotonin". It was not until 1958 that this problem of dual nomenclature was resolved, and the name "angiotensin" agreed upon by the two groups to replace hypertensin and angiotonin.

Plentl and Page (1943) by means of a kinetic study of the renin-renin activator reaction confirmed the enzymatic nature of renin. At about the same time Plentl, et al. (1943) by electrophoretic analysis and testing of separated components showed that renin activator was in the alpha-2 globulin fraction of plasma.

Much effort was devoted to the determination of the structure of the polypeptide responsible for the pressor response. After a great deal of experimental effort was expended toward the purification of angiotensin by chromatographic and countercurrent distribution techniques, the studies culminated in the work of Elliott and Peart (1956) who determined the amino acid sequence of bovine angiotensin to be as follows:

H•asp-arg-val-tyr-val-his-pro-phe-his-leu•OH

Utilizing similar techniques Skeggs et al. (1954) discovered that angiotensin actually existed in two forms which they identified as angiotensin I and II. They further found that angiotensin I was converted to angiotensin II by an enzyme in plasma which they called "plasma converting enzyme". In a subsequent communication Skeggs et al. (1956) reported that angiotensin II was a potent vasoconstrictor when administered into an isolated rat kidney perfused with physiological saline, while angiotensin I was virtually ineffective in their preparation. Consequently, they concluded that angiotensin I must be

very rapidly converted to II in the body. By the use of chromotography on ion exchange columns Lentz et al. (1956) demonstrated that angiotensin II was composed of equimolar amounts of the amino acids asp, pro, val, ileu, tyr, phe, hist, and arg. Therefore, they concluded that the converting enzyme broke the phenylalanyl-histidine bond forming angiotensin II and histidylleucine. Skeggs et al. (1956) then confirmed the expected amino acid sequence of angiotensin II to be asp-arg-val-tyr-ileu-hist-pro-phe.

The following scheme summarizes the mechanism of angiotensin formation as completely as is understood at the present time.

Renin Substrate
(Angiotensinogen)

↓ Renin

H•asp-arg-val-tyr-ileu-his-pro-phe-his-leu•OH
(Angiotensin I)

↓ Converting enzyme

H•asp-arg-val-tyr-ileu-his-pro-phe•OH
(Angiotensin II)

The next major step in the development of the renin-angiotensin system was provided by Bumpus, et al. (1957) who successfully synthesized isoleucyl^{5}-angiotensin II. Following the successful synthesis of the polypeptide, it became possible to make substitutions within the peptide chain which then led to the determination of the active radicals and the spatial configuration of the angiotensin II molecule. For a résumé of the work and the results obtained in these studies the reader is referred to the review by Page and Bumpus (1962).

b) Site of renin formation and factors controlling secretion.

In view of the fact that the kidney was established as the organ responsible for the production of renin, it then became of importance to attempt to determine the intrarenal site at which the enzyme was formed.

This intrarenal site has been established almost beyond question to be the cells of the juxtaglomerular apparatus (Deane and Masson, 1951; Cook and Pickering, 1958; Tobian et al., 1959; Pitcock, J. A., 1959; Edelman and Hartroft, 1961; Kuhn et al., 1961; Bing and Kazimierczak, 1962; Hartroft et al., 1964).

Following Goldblatt's original demonstration of experimental renal hypertension it was widely accepted that the stimulus for renin secretion was ischemia of the renal tissue. This concept persisted for many years, primarily due to the fact that the majority of investigators concerned themselves with the idea of implicating the renin-angiotensin system in the etiology of hypertension rather than investigating the normal control of the system. It is interesting to note the tremendous effort which was made to attach pathological significance to the system before its normal physiological role had been established.

Sapirstein et al. (1941) were the first to postulate that the renin-angiotensin system might function as a homeostatic mechanism to maintain normal blood pressure. Their conclusion was derived from the observation that upon hemorrhaging an animal they were able to demonstrate increased amounts of renin

by assaying plasma of the hemorrhaged dogs on isolated guinea pig ileum. The fact that blood levels of angiotensin are elevated following hemorrhage has subsequently been confirmed by several groups of investigators (Hamilton and Collins, 1941; Hamilton and Collins, 1942; Huidobro and Braun-Menendez, 1942; Dexter et al., 1943; Mikasa and Mason, 1961; Scornik and Paladini, 1964). However, it has been reported that the kidney content of renin is unaltered following hemorrhage (Gross et al., 1964), or following hypotension produced by ligation of the portal vein (Taquini et al. 1964). These latter observations are somewhat surprising, since the evidence showing elevated blood levels of angiotensin following these procedures would seem to be well substantiated. Therefore, if one is to accept the evidence indicating that the renal content of renin is unaltered, it becomes necessary to postulate that the rate of synthesis of renin by the renal cells is capable of adapting to match the rate of secretion. Whether or not this postulate is true remains to be proven.

The exact mechanism by which hypotension leads to increased blood levels of renin is still a subject of great controversy. Because Goldblatt was able to induce hypertension in dogs by partially obstructing the renal artery which effected renal ischemia and which, in turn, promoted the release of renin, it was naturally assumed that hypotension as well led to renin release by causing ischemia of the renal tissue. This concept was, however, disputed by Huidobro and Braun-Menendez (1942). They were unable to provoke renin release by the injection of KCN in doses which they believed would depress oxygen consump-

by the renal tissue. They also ventilated dogs with various gas mixtures low in oxygen or high in carbon dioxide, and again were unsuccessful in promoting renin release.

Further evidence against the concept of ischemia being responsible for renin release was presented by Divry (1951), who prepared dogs in such a way that the kidney could be perfused with either arterial or venous blood at the same perfusion pressure. She found that regardless of whether she used arterial or venous blood to perfuse the kidney no renin was released as long as the perfusion pressure was maintained within normal limits. However, renin was released if perfusion pressure was lowered, and again whether arterial or venous blood was utilized for perfusion was of no consequence. Skinner, et al. (1964) performed three experiments in which the arterial oxygen saturation was lowered from 50 to 70 per cent of control and were unable to show evidence of renin release. Thus they also concluded that anoxia could not be the stimulus for renin release. They further state that the renal tissue extraction of oxygen did not change when the arterial oxygen saturation was lowered so it is doubtful that the tissue was actually anoxic. In other experiments, Skinner et al. (1963, 1964) demonstrated that it is possible to initiate renin release by decreasing renal perfusion pressure without decreasing renal blood flow. Under these circumstances it is difficult to visualize tissue hypoxia unless intrarenal shunting of blood occurs which then leads to hypoxia at a critical site.

A decrease in renal perfusion pressure may in itself be a stimulus for renin secretion if a baroreceptor mechanism is

involved. The concept that renin secretion is regulated via a baroreceptor mechanism was first suggested by Kohlstaedt and Page (1940). They then postulated that the receptor was most sensitive to alterations in pulse pressure rather than changes in perfusion pressure per se but have recently retracted this hypothesis. (Skinner et al. 1964). They now feel that the stimulus is in fact a decrease in the mean renal perfusion pressure, rather than a change in the pulse pressure. Thus the conclusions of Skinner et al. and of Divry (1951) (who also concluded that the kidney contained a baro-sensitive device which was activated by a decreased perfusion pressure to stimulate the release of renin) are in agreement.

Tobian (1962) also postulated that renin secretion was controlled by a stretch receptor which he believed to be in the afferent glomerular arteriole. This conclusion was based on the fact that in nine conditions, in which either a decrease in pressure in the renal arterial bed or a reduction in blood volume in the renal arterial bed occurred, an increase in granulation of the juxtaglomerular cells was noted. Conversely in eight conditions in which pressure in the arteries was increased or blood volume was increased a decrease in granulation was observed. He believed that an increased granulation was associated with an increased secretion and decreased granulation with decreased secretion.

Thus there is attractive evidence that the primary hemodynamic alteration responsible for the secretion of renin from the kidney is a decreased renal perfusion pressure rather than tissue hypoxia or an alteration in pulse pressure. The next

question is whether the decreased renal perfusion pressure is itself the stimulus for renin secretion or whether the decrease in perfusion pressure leads to some other change in renal function which is the trigger to renin release.

A number of investigators now believe that perfusion pressure only indirectly regulates renin secretion. The hypothesis has been advanced that the concentration of sodium or the osmolarity of the fluid within the distal tubule is the direct controlling mechanism of renin secretion (Gross et al. 1964). Gross has advanced this hypothesis primarily on the observation that an increased concentration of renin within the kidneys and the peripheral blood of animals subjected to various experimental procedures such as salt loading, salt deprivation, adrenalectomy, unilateral renal artery occlusion with and without contralateral nephrectomy, etc. does not correlate with blood pressure, but it does correlate reasonably well with hypo-osmolarity of the fluid in the distal tubules. This hypothesis has also received support by Vander (1964), who has provided experimental evidence which indicates that the sodium concentration at the level of the macula densa may be the stimulus for renin release (a decreased sodium concentration bringing about an increased secretion and vice versa). This theory is based upon the observation that the amount of renin found in the renal venous effluent shows a good inverse correlation with sodium excretion. That is, as the sodium excretion decreases the renin concentration increases. If a clamp is applied to the aorta proximal to the bifurcation of the renal arteries and tightened so as to decrease renal perfusion pres-

sure to 90 mm of Hg, sodium excretion is decreased and simultaneously renin secretion is increased. However, if the animal is treated with a diuretic agent before the clamp is tightened no release of renin or decrease in sodium excretion can be demonstrated following constriction. Also, if the diuretic is administered following aortic constriction renin secretion is then reduced, and sodium excretion increased.

Vander's evidence would appear to provide strong support to the hypothesis that the physiological role of the renin-angiotensin system may be concerned with the regulation of electrolyte balance. This hypothesis has been presented by a number of investigators who have shown that angiotensin is capable of stimulating the release of aldosterone from the adrenal gland (Laragh, 1962; Mulrow and Ganong, 1962; Ganong et al., 1962; Davis, 1963).

For many years it was accepted that innervation of the kidney was not involved in renin secretion. This conclusion was primarily due to the many studies which demonstrated that renin could be released from either isolated kidneys or transplanted kidneys in which all innervation had been destroyed. However, since methods of assaying renin were rather unquantitative it was impossible to detect whether or not dennervation altered the magnitude of secretion. Recently Taquini et al. (1964) have demonstrated that three weeks following dennervation of the rat kidney, the renin content of the dennervated kidney is reduced as compared to the contralateral innervated kidney of the same animal. They conclude that this indicates that there must be an interaction between the nervous system and the

renin-angiotensin system. Bunag et al. (1964) have provided somewhat more convincing evidence of a nervous control of renin secretion by showing that renin could be released by bleeding an animal slowly so as not to decrease arterial blood pressure or renal blood flow. The release could be abolished or greatly reduced by the ganglion blocking agent tetraethylammonium or by the local application of Lidocaine to the renal hilus.

c) Effect of angiotensin on the vasculature.

Friedman et al. (1938) using a crude extract of the renal cortex attempted to determine the site of action of what they called renal pressor substance. They found that complete transection of the spinal cord in the cervical region increased the systemic pressor response evoked by the crude extract, and that evisceration had no significant effect on the response. They noted that following administration of the extract limb volume was decreased, and that kidney volume was initially decreased followed by an increase. They observed irregular effects when the extract was administered into an isolated dog's tail perfused with Tyrodes solution, but noted a well-sustained pressor response when the tail was perfused with blood. They, therefore, concluded that the pressor material (which was in all probability renin), was acting upon both splanchnic and peripheral vessels independent of the central nervous system.

The fact that angiotensin causes constriction of the smooth muscle of the arterioles has been rather universally accepted. In fact, angiotensin is reputed to be the most po-

tent vasoconstrictor known at the present time, having several fold the potency of norepinephrine (Finnerty, 1962).

The action of angiotensin on various regional resistances is not consistent. For example, Mandel and Sapirstein (1962) found that when angiotensin was infused into a rat the cerebral vascular resistance was reduced or unchanged, heart and lungs unchanged, spleen and adrenal increased minimally, carcass, skin and gut increased somewhat more, and kidney increased 100-200 per cent. From these observations they were led to conclude that the overall effect of angiotensin is to shunt blood away from the kidneys to the organs most concerned with the immediate response of the organism to an emergency.

In addition to the study of Mandel and Sapirstein, Rose et al. (1962) and Bianchi et al. (1960) have also shown that angiotensin has no effect on pulmonary vasculature as opposed to norepinephrine which has a direct vasoconstrictor action. Bianchi et al. demonstrated also that angiotensin produces a marked decrease in coronary flow.

Although angiotensin was believed for many years to be simply a direct acting vasoconstrictor, recent evidence indicates that the action of angiotensin upon smooth muscle may not be as uncomplicated as it was thought to be. Khairallah and Page (1961) reported that pentolinium, chlorisondamine, and TEA, ganglion blocking agents, did not reduce the response to angiotensin on the intestinal smooth muscle, but morphine and doses of nicotine large enough to produce depolarization blockade did. In view of these observations they postulated that angiotensin does not act by releasing acetylcholine from

the preganglionic neurons, but probably by combining with a "non-acetylcholine" receptor site on the cell body of the post-ganglionic neuron. Robertson and Rubin (1962) using isolated intestines of rabbit and guinea pig reported that the contraction produced by angiotensin could be potentiated by BW284051, a true chlinesterase inhibitor, and could be markedly attenuated by atropine or botulinum toxin. Consequently, they concluded that at least part of the response produced by angiotensin was due to the release of acetylcholine.

Additional evidence for an indirect component in the vasoconstricting action of angiotensin has been put forth by Bicherton and Buckley (1961) and by Zimmerman (1962). Bicherton and Buckley using a cross-circulation technique, demonstrated that when angiotensin was given to the head of the recipient animal, a pressor response was elicited in the body of the recipient. The response was abolished by the administration of the alpha adrenergic blocking agent, piperoxan, to the recipient's body. Vasopressin, unlike angiotensin, produced vasoconstriction only in the donor's body when administered to the head of the recipient. They concluded that angiotensin has a dual mechanism of action; a direct vasoconstriction not amenable to blockade by piperoxan, and a central hypertensive effect probably due to stimulation of central sympathetic structures which lead to an increased peripheral sympathetic discharge.

Zimmerman's work reveals that in perfused hindquarters of the dog, acute sympathectomy produces a significant reduction of the vasoconstrictor action of locally injected angiotensin

while the response evoked by norepinephrine is unchanged. Administration of the ganglion blocking agent, hexamethonium, or transection of the cervical spinal cord produced results comparable to those obtained by sympathectomy. Electrical stimulation of the peripheral end of the cut sympathetic trunk was shown to restore the vasoconstrictor action of angiotensin, whereas infusion of norepinephrine failed to restore the response. This evidence was interpreted to indicate that a portion of the vasoconstricting action of angiotensin is dependent upon normally functioning sympathetic nerves.

McCubbin and Page (1963) reported observations similar to those of Zimmerman. They observed that the pressor response evoked in the perfused mesenteric bed by occlusion of the common carotid arteries, was enhanced during an infusion of angiotensin. They further demonstrated that the interaction between angiotensin and the sympathetic nervous system must be occurring at the peripheral terminals, since splanchnic nerve recordings failed to show any significant change during the angiotensin infusion. Benelli et al. (1964) also reported similar findings with the isolated vas deferens preparation. Thus it would appear that angiotensin definitely possesses some property which allows it to augment the activity of the sympathetic nervous system in addition to its capacity to directly evoke vasoconstriction.

Folkow et al. (1961) using the technique developed by Mellander (1960) studied the effects of angiotensin on consecutive vascular segments in the hindlimb of the cat. They found, in accord with others, that angiotensin was more potent

than norepinephrine on resistance vessels, but norepinephrine far exceeded angiotensin in constricting the capacitance vessels. Thus they concluded that the angiotensin receptors were located only in resistance vessels, while those for norepinephrine were located both in resistance and capacitance vessels. Haddy et al. (1962) observed similar effects on both the forelimb and intestinal vascular beds of the dog. Utilizing isolated strips of mesenteric veins, Sparks (1963) also observed that angiotensin caused far less constriction than did norepinephrine. Kot et al. (1964) using a system by which the left ventricle was completely bypassed, and in which a reservoir system was interposed in the venous return to the heart, found that an injection of 2 mcg/kg of norepinephrine produced an increase in reservoir volume which amounted to about 8.5 per cent of the intravascular volume. On the other hand, an equiweight injection of angiotensin produced no increase in reservoir volume, in fact, on an average the reservoir volume decreased. They were also unable to obtain any evidence that angiotensin was able to constrict veins by directly applying the agent to an isolated venous segment.

In contrast to the aforementioned reports is a recent communication by Emerson et al. (1965). These investigators used the technique of recording pressures from consecutive vascular segments in both constant flow and natural flow forelimb preparations, and found that angiotensin caused a potent venoconstriction when compared to norepinephrine either on a weight or molar basis. For example, in the dog angiotensin was observed to be three times as potent as norepinephrine on an equimolar basis.

B. Statement of the problem

The present study arose out of an earlier study on hemorrhagic hypotension, in which it was noted that a progressive slow blood loss continued long after a fixed level of hypotension had been established. This secondary blood loss was presumptively non-adrenergic in origin since the sympathetic system is believed to be rapidly and maximally activated when the systemic blood pressure falls below 80 mm of Hg. Lum, B. K. B. (Doctoral Dissertation, University of Michigan, 1956) found that the further slow blood loss which occurs during controlled hypotension cannot be totally accounted for by hemodilution, and thus concluded that it must be due to progressive vasoconstriction. The present investigation was undertaken to determine the possible importance of the kidney in this response.

A second major aspect of the problem was to evaluate whether or not the sympathetic nervous system physiologically interacts with the renin-angiotensin system. Preliminary reports of this investigation have been presented (Beck, L., 1965; DuCharme, et al., 1965).

CHAPTER II

MATERIALS AND METHODS

A. Controlled hypotension

Healthy mongrel dogs of either sex, selected to weigh between 8 and 14 kg were used in this investigation. The dogs were anesthetized by the intravenous administration of 30 mg/kg of sodium pentobarbital, and following the operative procedures received 5 mg/kg of sodium heparin to prevent coagulation of the blood. In all cases, two experiments were performed simultaneously. One of the animals used was bilaterally nephrectomized and the other sham-operated for nephrectomy. The two experiments were performed on the same day to minimize the possibility of such uncontrollable variables as source of animal supply, environmental conditions, diet, etc. having a greater influence upon the results obtained from one group than those obtained from the other.

Controlled hypotension was accomplished in these experiments by the use of a blood pressure stabilization system which has been described in detail by Beck (1958). Figure 1 illustrates a diagram of the system. The system consists of a pressure source which supplies a pressure head to the pressure control reservoir and the stabilization reservoir which are connected in parallel. The pressure throughout the system (P_1) is determined by the hydrostatic pressure applied by the column of water on the outflow of air from the dip tube in the pressure control reservoir. A pressure cylinder containing a mixture of 95 per cent oxygen and 5 per cent carbon dioxide provided the pressure source.

FIGURE 1

SCHEMATIC DIAGRAM OF THE SYSTEM
UTILIZED FOR BLOOD PRESSURE STABILIZATION

See METHODS for description

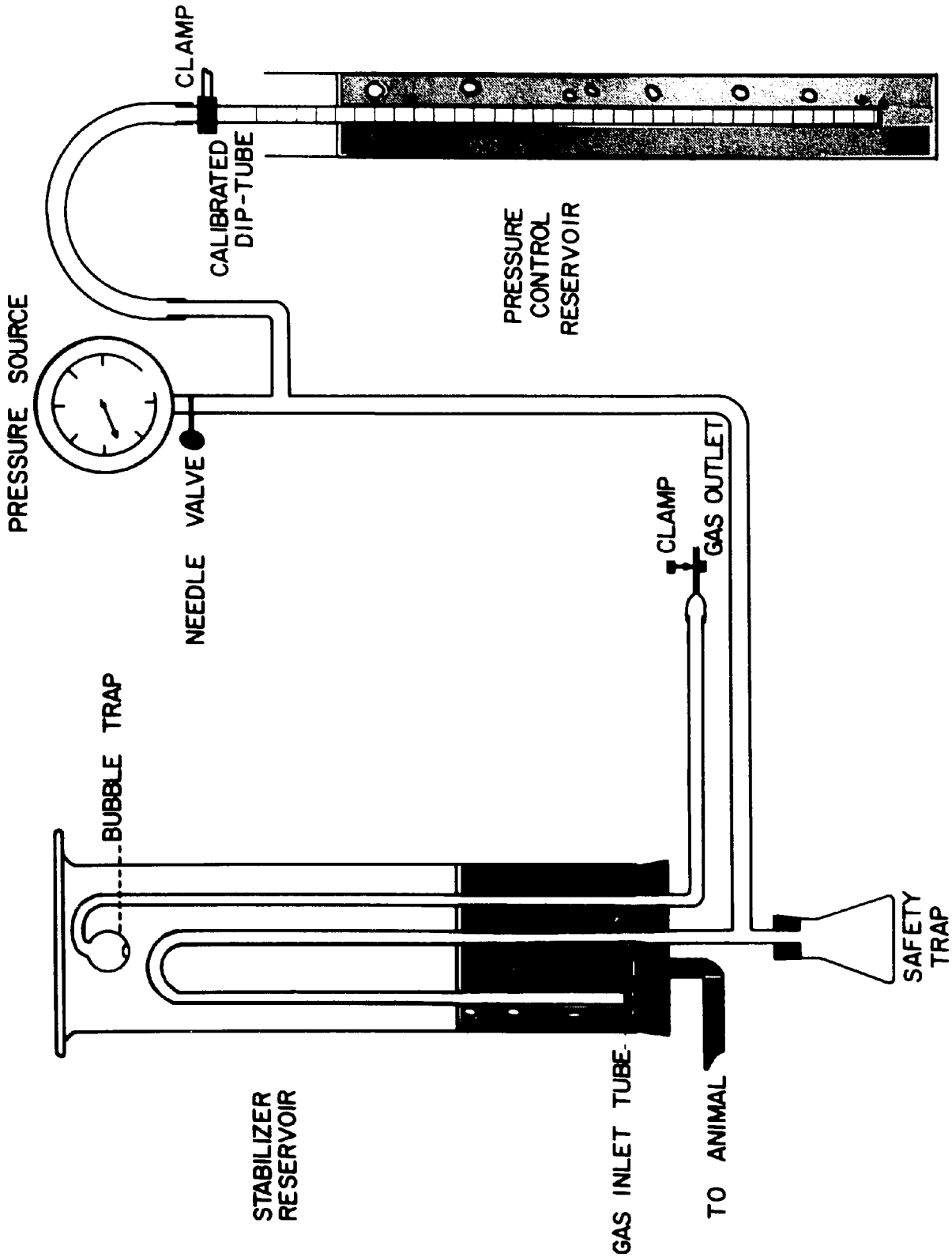

CLAMP
CALIBRATED DIP-TUBE
PRESSURE CONTROL RESERVOIR
PRESSURE SOURCE
NEEDLE VALVE
CLAMP
GAS OUTLET
BUBBLE TRAP
SAFETY TRAP
STABILIZER RESERVOIR
GAS INLET TUBE
TO ANIMAL

FIGURE 1

The major advantage of this system over other methods frequently used in controlled hypotension experiments is that it allows precise control of the arterial pressure regardless of the volume of blood in the reservoir, and without having to continually change the position of the blood reservoir. This is achieved primarily by means of the "U" shaped gas inlet tube in the stabilization reservoir. At a time when the reservoir contains no blood the gas pressure in the reservoir (P_3) is equal to the hydrostatic pressure (P_1) exerted on the outflow of air from the dip tube in the control reservoir. As the blood level rises above the tip of the gas inlet tube, the outflow of air from this tube must overcome the hydrostatic pressure of the blood (P_2) above the tip of the inlet tube, therefore, the effective gas pressure in the reservoir (P_3) will decrease by exactly the same amount as the hydrostatic pressure (P_2) exerted by the column of blood. Consequently, irrespective of the amount of blood in the reservoir $P_2 + P_3$ will always be equal to P_1 if gas is bubbling through the blood in the stabilizer reservoir and from the dip tube in the pressure control reservoir, and if the gas outflow tube from the stabilization reservoir is open sufficiently to allow the gas above the blood to escape as the blood level rises. This system also has the advantage that the shed blood is continuously agitated and oxygenated by the gas mixture bubbling through it. Excessive frothing of the blood is prevented by restricting the egress of gas from the gas outflow tube, which effectively reduces the rate at which the gas bubbles through the blood.

The stabilization pressure used in the majority of the experiments presented in this investigation was 45 mm of Hg. When pressures higher than this were desired zinc bromide was substituted for water in the pressure control reservoir. The desired hypotensive level was achieved in the experimental animal by allowing the animal to bleed from a polyethylene cannula of maximal diameter inserted through the femoral artery into the lower abdominal aorta, and connected by rubber tubing to the stabilizer reservoir. The blood pressure of the animal was continually monitored by a mercury manometer attached to a T-tube inserted in the rubber tubing connecting the animal to the reservoir, to assure the investigator that the proper level of hypotension was achieved and maintained throughout the experiment.

At the outset of the experiment a pinch clamp was removed from the rubber tubing between the animal and the reservoir, and the animal allowed to hemorrhage as rapidly as possible into the reservoir. The volume of blood was then read directly and recorded at 1 minute intervals for the first 15 minutes, 5 minute intervals for the next 15 minutes, and 10 minute intervals for the remainder of the experiment. Blood samples were taken from the animal for hematocrit determinations immediately prior to the onset of hemorrhage and at 2, 5, 10, 30, 60, 90, and 120 minutes after. The animals prehemorrhage blood pressure was also recorded. At the termination of the experiment the animals were sacrificed by administering a lethal dose of the anesthetic agent.

B. Surgical procedures

The surgical procedures performed on the animals used for the hypotensive experiments were all accomplished by the use of a scalpel, and completed at least two hours prior to the onset of the experiment. Care was taken to ligate all bleeders which appeared during the surgery.

1. Nephrectomy.

The kidneys were approached retroperitoneally through longitudinal incisions in both the left and right flank regions. The renal artery was then exposed and securely ligated. Before ligating the renal vein the kidney was allowed to passively expel as much of its blood content as possible, the renal vein and the ureter were then ligated. The kidney was freed from the surrounding tissue, the artery, vein, and ureter sectioned, and the kidney removed.

2. Sham-operation.

The surgical procedure used for the sham-operations was identical to that used for nephrectomy. The kidney was approached retroperitoneally and located, however, it was not isolated from the surrounding tissue and was manipulated as little as possible. Great care was taken not to interrupt either the circulation or the innervation of these kidneys.

3. Tracheotomy and exposure of femoral artery.

A midline incision was made in the neck of all dogs and a tracheotomy performed to insure an adequate airway throughout the experiment.

The femoral artery was approached through an incision in the medial aspect of the thigh. The artery was exposed and

isolated from the accompanying nerve and vein for a distance of about 1 inch from the point at which it emerges dorsal to the inguinal ligament.

C. Spinal anesthesia

A spinal needle was inserted through the skin and muscle overlying the upper lumbar vertebrae and finally through the intervertebral space into the subarachnoid space. The appearance of clear cerebrospinal fluid indicated proper placement of the needle. Anesthesia was instituted by administering 3-4 cc of 10 per cent procaine hydrochloride through the needle. Complete cessation of respiratory movements was used as the criterion for adequate spinal anesthesia; therefore, these animals were artificially respired with a Harvard[1] respirator. A stroke volume of 10-15 cc/kg of room air at a rate of 15-20 strokes per minute closely approximated the animals normal respiratory exchange. In the hypotension experiments, spinal anesthesia was initiated 30 minutes prior to the onset of hemorrhage.

D. Perfusion of the hindquarters

Again mongrel dogs of either sex were anesthetized with 30 mg/kg of sodium pentobarbital administered intravenously, and heparin sodium, 5 mg/kg, was given following the operative procedures to prevent coagulation of the blood.

The method used for perfusion of the hindquarters was that described by Beck (1961). Figure 2 illustrates a diagrammatic sketch of the system. The lower abdominal aorta was

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[1] Model 607 Harvard Apparatus Co., Dover, Massachusetts

FIGURE 2

SCHEMATIC DIAGRAM OF THE PERFUSION SYSTEM
UTILIZED FOR AUTOCIRCULATION OF THE HIND LIMBS

See METHODS for description

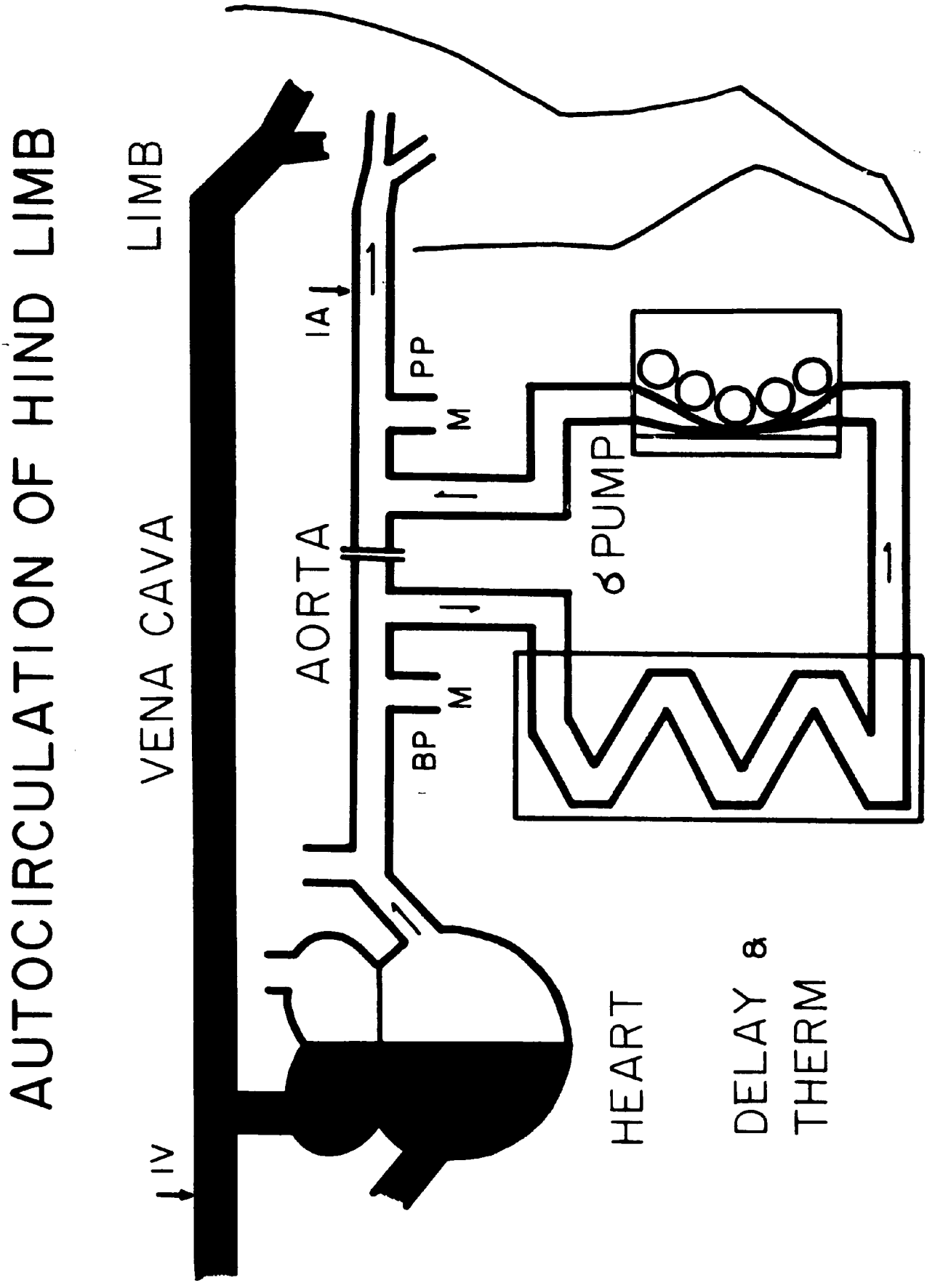

AUTOCIRCULATION OF HIND LIMB
LIMB
VENA CAVA
AORTA
IA
PP
M
PUMP
BP
M
IV
HEART
DELAY &
THERM

FIGURE 2

exposed retroperitoneally through a longitudinal incision made in the left flank of the dog with a hot wire cautery. The aorta was then ligated opposite the body of the third lumbar vertebrae. A constant flow Sigmamotor pump was used to pump blood from a large polyethylene cannula placed in the aorta proximal to the point of ligation to a second cannula placed in the aorta distal to the ligature. The systemic blood pressure was measured by a double-arm mercury manometer attached to a T-tube placed in the rubber tubing connecting the proximal aortic cannula to the pump, and recorded on a smoked paper kymograph. The perfusion pressure of the hindquarters was recorded similarly by a single-arm mercury manometer attached to a T-tube placed in the rubber tubing leading from the pump to the distal aorta. The blood flow to the hindquarters was adjusted so as to make the perfusion pressure equal to the systemic blood pressure. Adjustment of the blood flow was made possible by a Graham variable speed transmission attached to the pump. After initial adjustment the blood flow remained constant throughout the experiment, thus changes in perfusion pressure reflect in direct proportion, changes in peripheral resistance.

A midline incision was made in the neck to permit cannulation of the trachea and of the external jugular vein which was used for the intravenous administration of drugs. Intra-arterial administration of drugs to the hindquarters was executed by injecting the agents into the lumen of the rubber tubing attached to the cannula inserted into the distal portion of the aorta.

Electrical stimulation of the sympathetic trunks which were isolated in the mid-lumbar region, was accomplished by the use of Harvard bipolar shielded electrodes[1] and American Electronic Laboratory stimulators[2]. Similar equipment was also used to stimulate the sciatic nerve which was isolated as it emerges from the pelvis at the great sciatic notch.

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[1] #306, Harvard Apparatus Company, Dover, Massachusetts
[2] Model 104A, American Electronic Laboratories, Inc., Philadelphia, Pennsylvania

CHAPTER III

RESULTS

A. Bleeding volumes of sham-operated and bilaterally nephrectomized dogs subjected to severe hypotension.

1. Control animals.

The upper portion of Figure 3 illustrates the average bleeding volumes obtained from 11 sham-operated and 9 nephrectomized dogs when the arterial pressure was decreased from normal to 45 mm of mercury. The bleeding volumes of the sham-operated animals (solid circles) and those of the nephrectomized animals (open circles) were nearly identical during the first 4 minutes of hemorrhage. A difference between the two began to appear by the 5th minute of hypotension, and progressively increased until reaching a maximum at about 30 minutes. At this time the difference was 10.4 cc of blood per kilogram of body weight.

The average bleeding volumes accumulated in the reservoir at selected time intervals throughout the period of hypotension are shown in Table 1. The difference in bleeding volume between the sham-operated and the nephrectomized dogs was statistically significant only at the 30 minute recordings.

The secondary bleeding volumes (the difference betwen the recorded volume at 2 minutes and that recorded at subsequent times) were significantly different at all times throughout the period of sustained hypotension except at the two hour reading as shown in Table 2.

2. Animals with spinal anesthesia.

The barred circles in the lower portion of Figure 3

FIGURE 3

EFFECT OF NEPHRECTOMY ON BLEEDING VOLUMES OF CONTROL ANIMALS AND ANIMALS WITH SPINAL ANESTHESIA

Each point represents the average response of several animals. See Tables 1 and 3 and text for description.

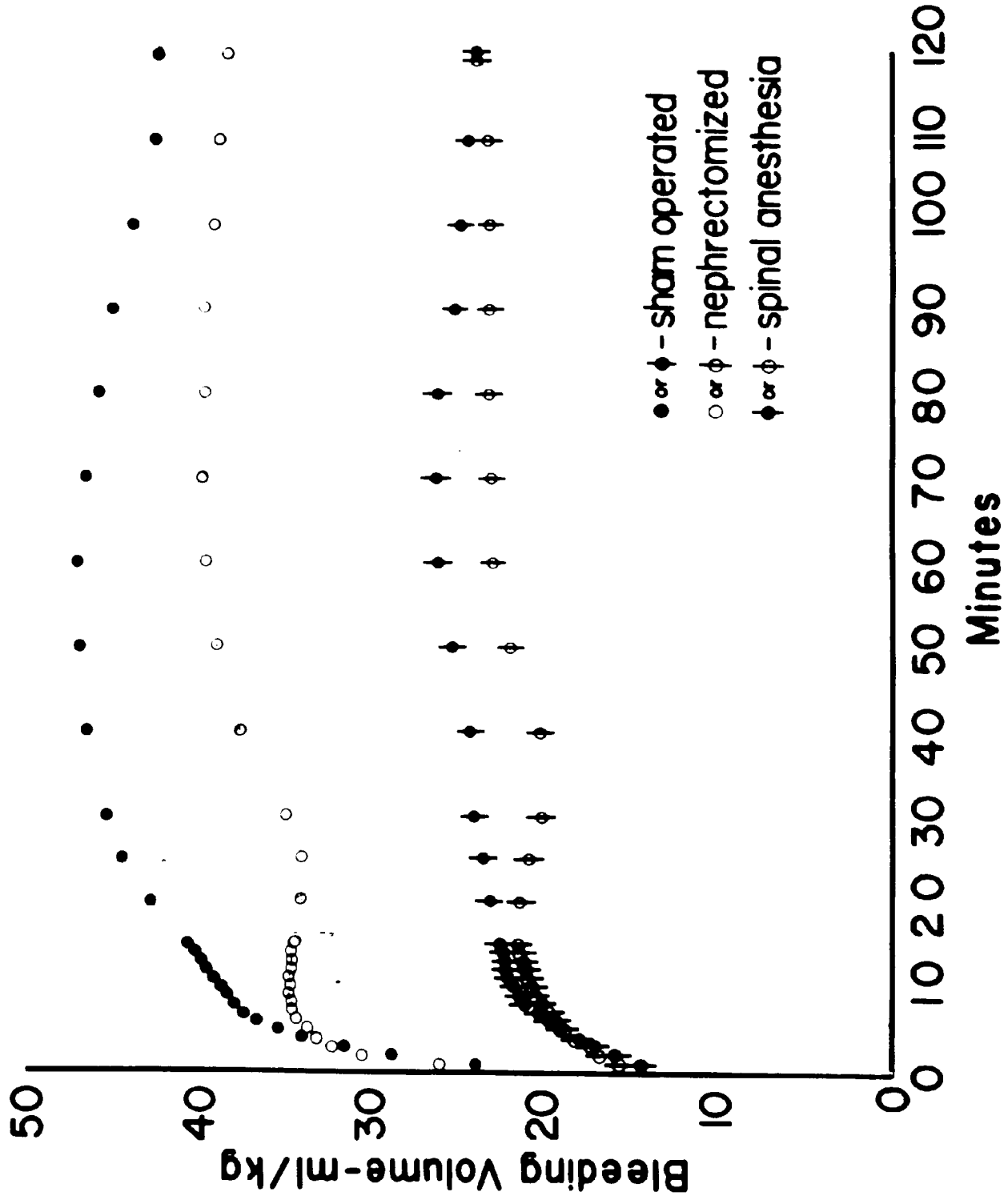

Bleeding Volume-ml/kg
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100 110 120
Minutes
● ∝ ♦ - sham operated
○ ∝ ♦ - nephrectomized
♦ ∝ ♦ - spinal anesthesia

FIGURE 3

TABLE 1

EFFECT OF NEPHRECTOMY ON BLEEDING VOLUMES
OF CONTROL ANIMALS

Treatment	Bleeding Volumes (ml/kg±S.E.) at Selected Times (mins.)						
	2	5	10	30	60	90	120
Sham-operated n=11	28.7±2.1	35.4±2.4	38.6±2.6	45.3±2.8	47.0±2.4	44.8±1.8	42.1±1.5
Nephrectomized n=9	30.4±2.2	33.5±2.8	34.5±3.3	34.9±3.4	39.5±2.8	39.5±2.7	37.7±2.6
Difference	-1.7	1.9	4.1	10.4*	7.5	5.3	4.4

* Difference is significant $p<.05$

TABLE 2

EFFECT OF NEPHRECTOMY ON SECONDARY BLEEDING VOLUMES OF CONTROL ANIMALS

Treatment	Secondary Bleeding Volumes (ml/kg±S.E.) at Selected Times (mins.)					
	5	10	30	60	90	120
Sham-operated n=11	6.7±0.8	9.9±1.1	16.6±1.3	18.3±2.3	16.1±2.4	14.2±2.2
Nephrectomized n=9	3.1±0.9	4.1±1.7	4.5±1.8	9.1±1.9	7.9±2.2	7.2±2.7
Difference	3.6	5.8	12.1	9.2	8.2	7.0
Significance of difference	$p<.01$	$p<.01$	$p<.001$	$p<.01$	$p<.05$	$p<.1$

illustrate the average bleeding volumes obtained from a series of 15 dogs which were subjected to complete spinal anesthesia 30 minutes prior to the onset of hypotension. The closed circles represent the average response of 8 sham-operated animals and the open circles that of 7 nephrectomized animals. It is readily apparent that the bleeding volumes of animals with spinal anesthesia are considerably less than those of the control dogs. It can be seen upon careful observation, however, that spinal anesthesia caused a greater reduction in the bleeding volume of sham-operated animals than of nephrectomized animals. At 30 minutes the difference between the nephrectomized animals with and without spinal anesthesia was 15 ml/kg, whereas the difference between the sham-operated groups with and without spinal anesthesia was 22 ml/kg.

In addition it can be seen from Figure 3 that spinal anesthesia decreased the difference in bleeding volume between the sham-operated and nephrectomized animals, the differences at 30 minutes being 3.9 ml/kg for the spinally anesthetized group (Table 3), and 10.4 ml/kg for the group with no spinal anesthesia (Table 1).

The secondary bleeding volumes were calculated for the spinally anesthetized group in the same manner as previously described for the control group. Figure 4 illustrates the graphic representation of the calculated secondary bleeding volumes for both groups of animals. The upper graph being that for the control animals, and the lower graph the animals which received spinal anesthesia.

The difference in secondary bleeding volume between the

TABLE 3

EFFECT OF NEPHRECTOMY ON BLEEDING VOLUMES
OF ANIMALS WITH SPINAL ANESTHESIA

Treatment	Bleeding Volumes (ml/kg S.E.) at Selected Times (mins.)						
	2	5	10	30	60	90	120
Sham-operated n=8	15.9±1.2	18.7±1.5	21.5±1.8	23.8±1.5	25.8±2.1	24.9±1.9	23.5±2.2
Nephrectomized n=7	16.7±1.5	18.6±1.3	20.5±1.8	19.9±2.2	22.6±1.4	22.7±1.4	23.5±1.5
Difference*	-0.8	0.1	1.0	3.9	3.2	2.2	0.0

* All differences are statistically insignificant

FIGURE 4

EFFECT OF NEPHRECTOMY ON SECONDARY BLEEDING
VOLUMES OF CONTROL ANIMALS AND ANIMALS WITH SPINAL ANESTHESIA

Each point represents the average response of several animals.
See Tables 2 and 4 and text for description.

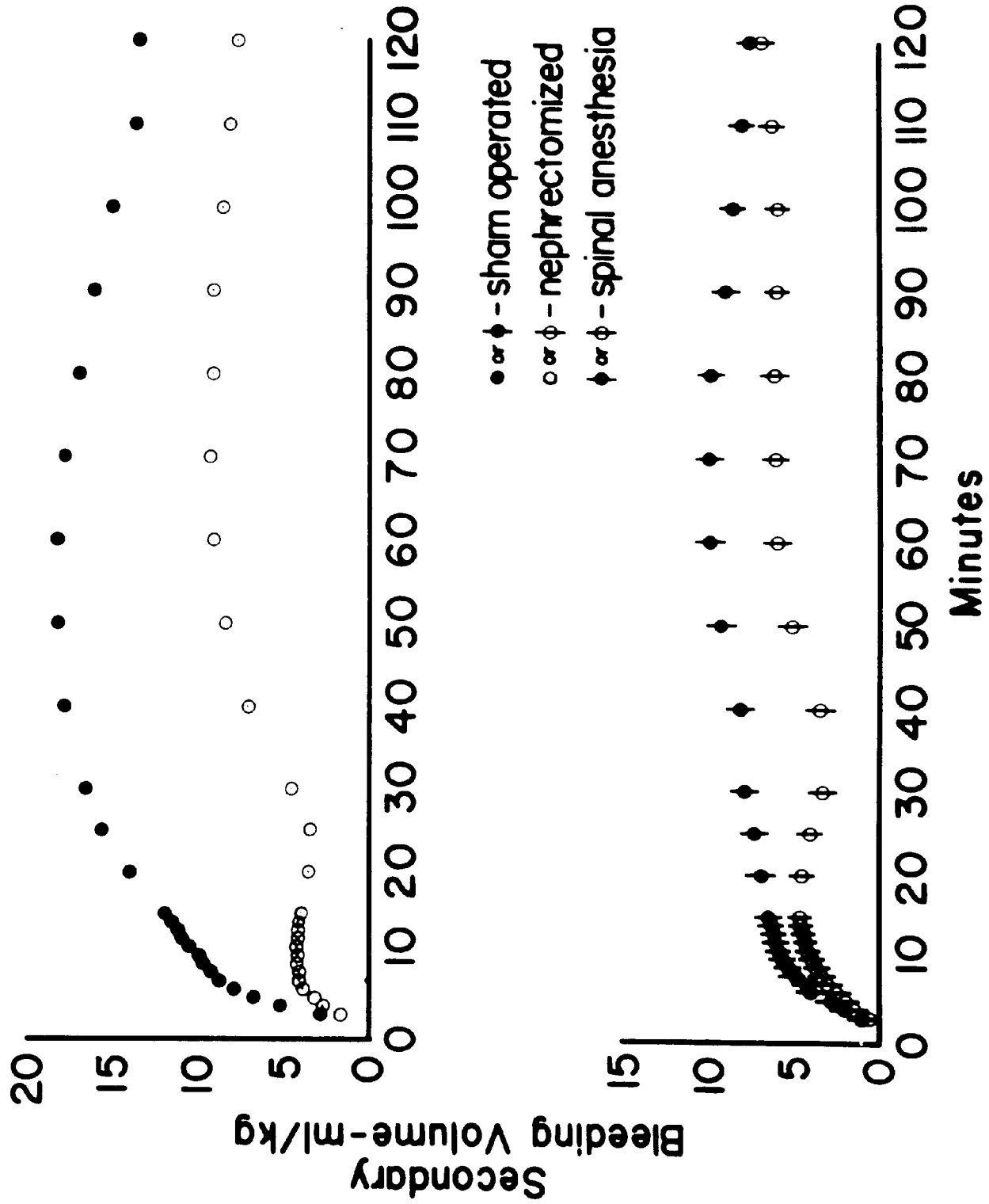

Secondary Bleeding Volume-ml/kg
20
15
10
5
0
0 10 20 30 40 50 60 70 80 90 100 110 120
●or♦-sham operated
○or◊-nephrectomized
♦or◊-spinal anesthesia
15
10
5
0
0 10 20 30 40 50 60 70 80 90 100 110 120
Minutes

FIGURE 4

sham-operated and the nephrectomized animals with spinal anesthesia is significant only at the 30 minute recording. (Table 4). In addition, it was determined by using a 2 x 2 factorial arrangement and the method of unweighted means (Snedecor, 1956), that at 30 minutes the difference between the control sham-operated and nephrectomized dogs is significantly greater ($p<.05$) than the difference between the sham-operated and nephrectomized dogs having spinal anesthesia.

In order to assess the completeness of spinal anesthesia throughout the period of hypotension, either a ganglion blocking agent or additional local anesthetic was administered before terminating the experiment, and the amount of reinfusion noted. In all of the experiments considered in the foregoing analysis reinfusion of blood was found to be either absent or negligible.

When the bleeding patterns of control and spinally anesthetized, nephrectomized animals were compared during the early minutes of hypotension it became apparent that the rate of bleed as well as the actual volume was depressed in the latter. The control nephrectomized animals reached a plateau in the bleeding curve at about 6 minutes, whereas the nephrectomized animals with spinal anesthesia did not reach a similar plateau until about 14 minutes.

3. Animals pretreated with phenoxybenzamine.

In this series of experiments all of the animals received 5 mg/kg of phenoxybenzamine (Dibenzyline) intravenously 2 hours prior to the onset of hypotension. Figure 5 illustrates the average bleeding volumes of 5 sham-operated (closed circles)

TABLE 4

EFFECT OF NEPHRECTOMY ON SECONDARY BLEEDING VOLUMES OF ANIMALS WITH SPINAL ANESTHESIA

Treatment	Secondary Bleeding Volumes (ml/kg±S.E.) at Selected Times (mins.)					
	5	10	30	60	90	120
Sham-operated n=8	2.8±0.6	5.9±0.9	7.9±1.4	9.9±1.9	9.0±1.9	7.6±2.3
Nephrectomized n=7	2.0±0.1	3.9±0.6	3.3±1.5	6.0±1.6	6.1±1.7	6.9±1.1
Difference	0.8	2.0	4.6*	3.9	2.9	0.7

* Difference is statistically significant $p<.05$

FIGURE 5

EFFECT OF NEPHRECTOMY ON BLEEDING VOLUMES
OF ANIMALS PRETREATED WITH PHENOXYBENZAMINE

Each point represents the average response of several animals. See text for description.

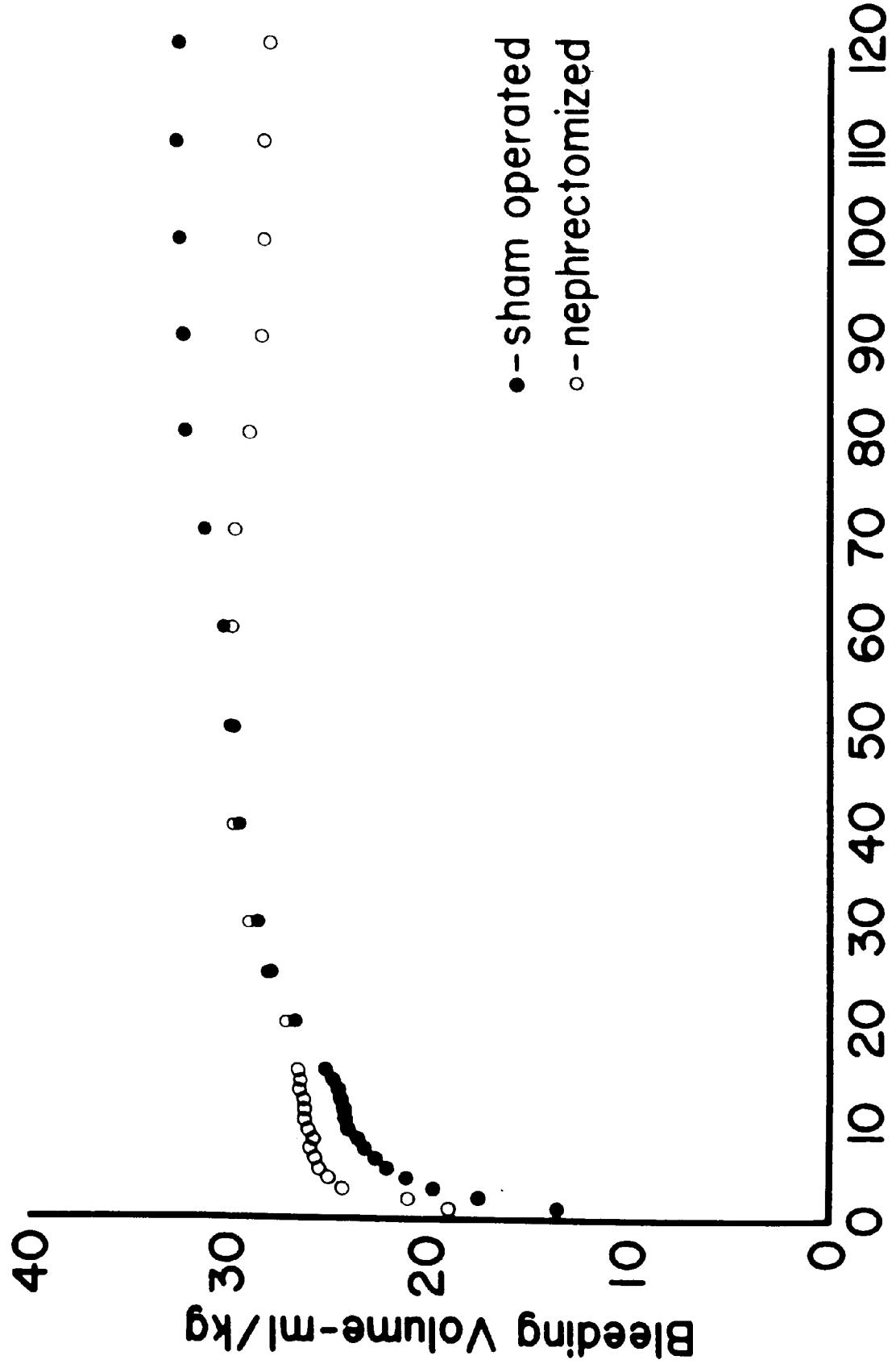

●-sham operated
○-nephrectomized
Bleeding Volume-ml/kg
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100 110 120
Minutes

FIGURE 5

and 5 nephrectomized animals (open circles) which received the alpha adrenergic blocking agent. The bleeding volumes of animals treated with phenoxybenzamine are greatly reduced compared to those of the control animals. In addition, Table 5 shows that the differences between the secondary bleeding volumes of the sham-operated and nephrectomized dogs treated with phenoxybenzamine are also less than those of the control animals. The differences between the secondary bleeding volumes of the shammed and nephrectomized animals did not become statistically significant until 1½ hours after induction of hypotension.

One additional observation worthy of mention is that when the phenoxybenzamine treated dogs were rendered hypotensive the rate at which blood accumulated in the reservoir was just as rapid as that observed in the untreated dogs, eventhough the animals treated with phenoxybenzamine bled considerably less than did the control animals.

4. Animals pretreated with guanethidine.

The dogs used in this phase of the present investigation were chronically pretreated with 30 mg/kg of guanethidine administered subcutaneously in two doses. An initial dose of 20 mg/kg was followed 2 days later by a second dose of 10 mg/kg. Animals were used for experimentation on the third day.

The results illustrated in Figure 6 represent data obtained from 11 sham-operated (closed circles) and 11 nephrectomized animals (open circles). The bleeding volumes of both the shammed and nephrectomized dogs pretreated with

TABLE 5

EFFECT OF NEPHRECTOMY ON SECONDARY BLEEDING VOLUMES OF ANIMALS PRETREATED WITH PHENOXYBENZAMINE

Treatment	Secondary Bleeding Volumes (ml/kg±S.E.) at Selected Times (mins.)					
	5	10	30	60	90	120
Sham-operated n=5	4.6±0.7	6.5±1.2	11.0±1.6	12.8±2.5	15.0±3.1	15.1±3.7
Nephrectomized n=5	3.4±1.1	4.2±1.6	7.0±2.0	7.8±1.4	6.4±2.1	6.0±2.2
Difference	1.2	2.3	4.0	5.0	8.6*	9.1

* Difference statistically significant $p<.05$

FIGURE 6

EFFECT OF NEPHRECTOMY ON BLEEDING
VOLUMES OF ANIMALS PRETREATED WITH GUANETHIDINE

Each point represents the average response of several animals. See Table 6 and text for description.

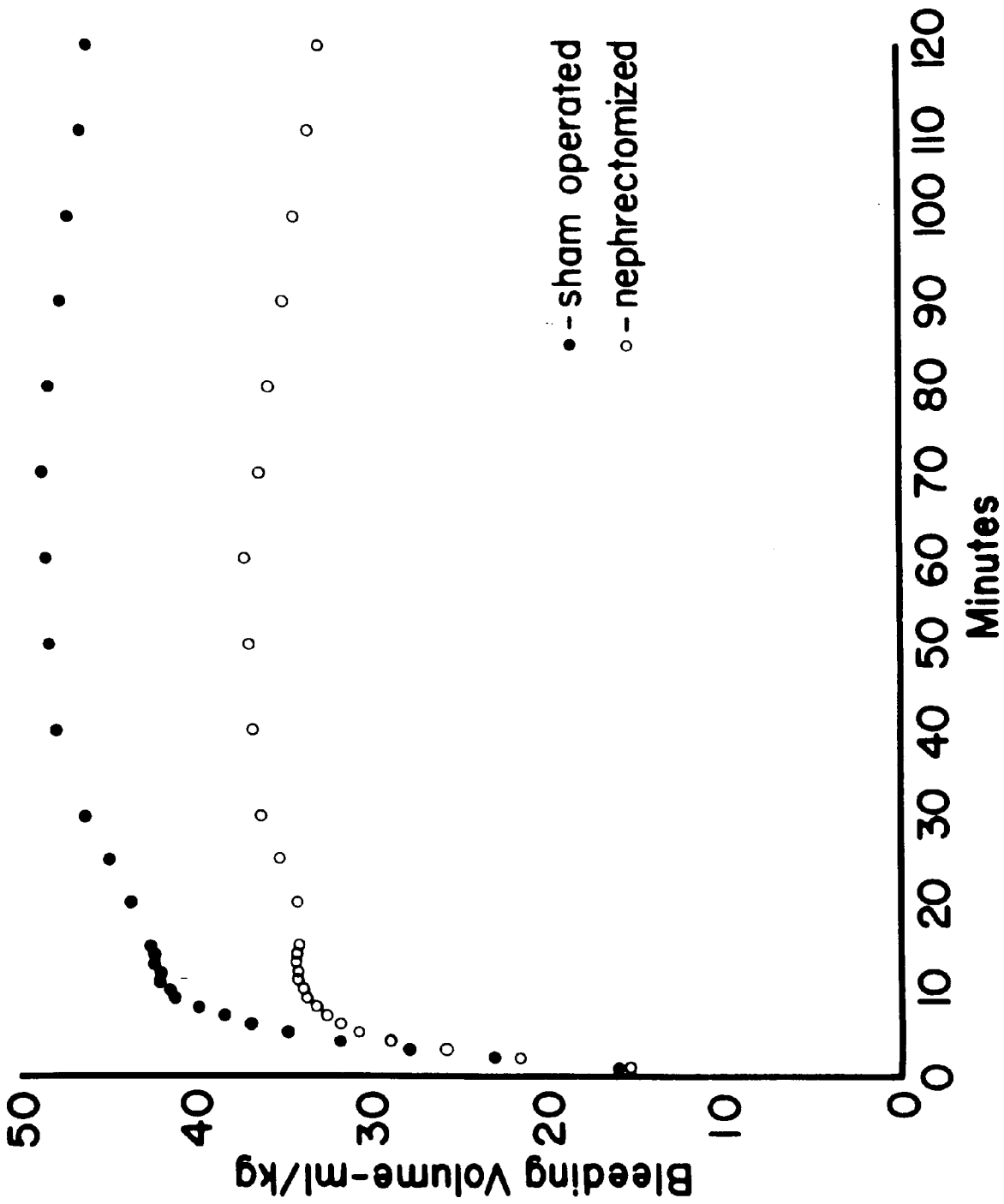

Bleeding Volume-ml/kg
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100 110 120
Minutes
•-sham operated
○-nephrectomized

FIGURE 6

guanethidine do not vary significantly from those obtained with the control animals, except at the 2 minute time interval (Table 6). The guanethidine treated nephrectomized dogs required 11-12 minutes before reaching a plateau in the bleeding curve.

B. Treatment interactions

In an attempt to shed some light on the seemingly paradoxical results obtained from the animals treated with guanethidine, another series of experiments was performed in which 17 dogs were utilized. Eight of these seventeen animals were treated with guanethidine in the same manner as before and 9 served as controls. None of the animals in this series was nephrectomized or sham-operated for nephrectomy, however, following the induction of anesthesia, time periods comparable to those used in previous experiments were allowed. After one hour of hypotension the animals were subjected to complete spinal anesthesia, and the volume of blood which reinfused into the animal over a 15 minute period was recorded. The results of these experiments are summarized in Table 7. Although spinal anesthesia induced a substantial reinfusion of blood by both groups of animals, the reinfusion by the normal dogs was significantly greater than that of the animals treated with guanethidine. The bleeding volumes of the two groups were nearly equal at the time the local anesthetic was administered, and were not significantly different than those of the sham-operated animals in the previous series at a comparable time.

In an additional 15 dogs, 8 treated with guanethidine and

TABLE 6

BLEEDING VOLUMES OF GUANETHIDINE TREATED ANIMALS
COMPARED TO THOSE OF CONTROL ANIMALS

Treatment	Bleeding Volumes (ml/kg S.E.) at Selected Times (mins.)						
	2	5	10	30	60	90	120
Control Sham-operated n=11	28.7±2.1	35.4±2.4	38.6±2.6	45.3±2.8	47.0±2.4	44.8±1.8	42.1±1.5
Guanethidine Sham-operated n=11	23.0±1.0	34.6±1.4	41.4±1.3	46.4±1.3	48.5±1.3	47.6±1.3	46.2±1.6
Difference	5.7*	0.8	-2.8	-1.1	-1.5	-2.8	-4.1
Control Nephrectomized n=9	30.4±2.2	33.5±2.8	34.5±3.3	34.9±3.4	39.5±2.8	39.5±2.7	37.7±2.6
Guanethidine Nephrectomized n=11	21.5±1.1	30.6±1.6	33.7±1.5	36.2±2.0	37.1±2.3	34.8±2.4	32.7±2.6
Difference	8.9**	2.9	0.8	-1.3	2.4	4.7	5.0

* Difference is statistically significant $p<.05$
** Difference is statistically significant $p<.01$

TABLE 7

EFFECT OF SPINAL ANESTHESIA ON VASCULAR CAPACITY OF GUANETHIDINE PRETREATED AND NORMAL DOGS

Guanethidine Pretreated Dogs		Normal Dogs	
Exp. No.	Reinfusion in 15 min. mls/kg	Exp. No.	Reinfusion in 15 min. mls/kg
1	9.2	1	15.0
2	14.6	2	26.0
3	14.3	3	19.0
4	18.9	4	34.0
5	24.5	5	33.9
6	5.6	6	25.4
7	18.8	7	18.8
8	16.5	8	25.0
		9	26.4
Average±S.E.	15.3±2.08*		24.8±2.17*

* Means significantly different ($p<.01$)

7 controls, the alpha adrenergic blocking agent, phentolamine (Regitine), was administered intravenously at a dose of 3 mg/kg also one hour after the onset of hypotension. The results of this series of experiments are shown in Table 8. During the 15 minute period immediately following the administration of phentolamine, an equal volume of blood was reinfused by both the guanethidine treated and the control animals. Thirty minutes after phentolamine, 6 dogs treated with guanethidine and 4 control animals were given spinal anesthesia. The data from these experiments are shown in Table 9. During the 15 minute period following anesthesia of the spinal cord the animals treated with guanethidine reinfused only a small quantity of blood, which was calculated to be insignificantly different than zero. The much greater reinfusion which occurred in the control animals was not only significantly different than zero, but was significantly greater than that of the guanethidine treated animals.

C. Effect of various drug treatments on pre-hemorrhage blood pressures

The control blood pressures of the various groups of animals, recorded immediately prior to the onset of hemorrhagic hypotension, are shown in Table 10. It can be seen that although the average blood pressures of the animals treated with spinal anesthesia, phenoxybenzamine, and guanethidine were lower than those of the respective control animals, the decrease was only slight.

D. Effect of various procedures and agents on the vasoconstrictor action of angiotensin in the perfused hindlimb of the dog

TABLE 8

EFFECT OF PHENTOLAMINE ON VASCULAR CAPACITY OF GUANETHIDINE PRETREATED AND NORMAL DOGS

Guanethidine Pretreated Dogs		Normal Dogs	
Exp. No.	Reinfusion in 15 min. mls/kg.	Exp. No.	Reinfusion in 15 min. mls/kg.
1	12.3	1	20.5
2	13.2	2	6.0
3	13.2	3	10.2
4	11.0	4	10.9
5	13.2	5	12.6
6	7.1	6	12.5
7	12.7	7	10.2
8	11.0		
Average ±S.E.	11.71 ± 0.73*		11.84 ± 1.66*

* Means not significantly different

TABLE 9

EFFECT OF SPINAL ANESTHESIA ON VASCULAR CAPACITY OF GUANETHIDINE PRETREATED AND NORMAL DOGS PREVIOUSLY TREATED WITH PHENTOLAMINE

Guanethidine Pretreated Dogs		Normal Dogs	
Exp. No.	Reinfusion in 15 min. mls/kg	Exp. No.	Reinfusion in 15 min. mls/kg
1	0.0	7	12.9
2	1.7	8	11.7
3	3.4	9	14.7
4	6.7	10	15.4
5	0.7		
6	12.1		
Average ±S.E.	4.1 ± 1.87*		13.67 ± .85**

* Not significantly different from zero ($p<.1$)
** Significantly greater than guanethidine treated ($p<.05$)

TABLE 10

EFFECT OF DRUG TREATMENTS ON
PRE-HEMORRHAGE BLOOD PRESSURES

Treatment	Average Blood Pressures (mm of Hg)			
	Control	Spinal anesthesia	Phenoxybenzamine	Guanethidine
Sham-operated	121±3.6 (11)*	107±6.0 (8)	102±4.6 (5)	109±2.3 (11)
Nephrectomized	129±4.0 (9)	118±9.6 (7)	115±3.3 (5)	105±4.8 (11)

* Figures in parentheses indicate number of animals

1. Interruption of adrenergic tone.

Twenty-nine experiments were performed in which the sympathetic nerves supplying the perfused vasculature of the hind extremities of the dog were decentralized by crushing the lumbar sympathetic trunks opposite the body of the third lumbar vertebrae. Control responses to the intra-arterial injection of norepinephrine and angiotensin were obtained prior to the decentralization, and again following the decentralization after the perfusion pressure had reached a stable level. The first two panels of the upper record illustrated in Figure 7 show a typical experiment in which this procedure was followed. The upper tracing is that of the perfusion pressure, the middle tracing the systemic blood pressure, and at the bottom of the record the zero reference line and time marker in minutes. It can be seen that following section of the sympathetic nerves, performed between the first and second panel, the pressor response evoked by the intra-arterial injection of angiotensin is attenuated as compared to the control response shown in the first panel. In contrast, the response produced by norepinephrine was virtually unchanged in this experiment. Although the response to angiotensin was almost always decreased following denervation, the alteration of the norepinephrine response was observed to vary from slightly increased, to no change, to slightly decreased. In the 29 experiments performed, the average response to norepinephrine following decentralization of the sympathetic nerves was reduced to 88 per cent of the control response, while that to angiotensin was reduced to 56 per cent of the control response.

FIGURE 7

EFFECT OF NEUROGENIC ACTIVITY
ON THE VASOCONSTRICTOR ACTION OF ANGIOTENSIN

Upper record. 26.0 kg female dog. Perfusion at left hindlimb. Top tracing: perfusion pressure. Bottom tracing: systemic blood pressure. Scale at the left measures systemic blood pressure (BP) and perfusion pressure PP in mm of Hg. Time base 1 minute.

Angio - Angiotensin II amide (Hypertensin, Ciba)

NE - norepinephrine

Symp-x- decentralization of left lumbar sympathetic trunk

Lower record. 12.0 kg male dog. Legend same as above.

Sci-x- section of sciatic nerve

$ sci - stimulation of distal end of cut sciatic nerve

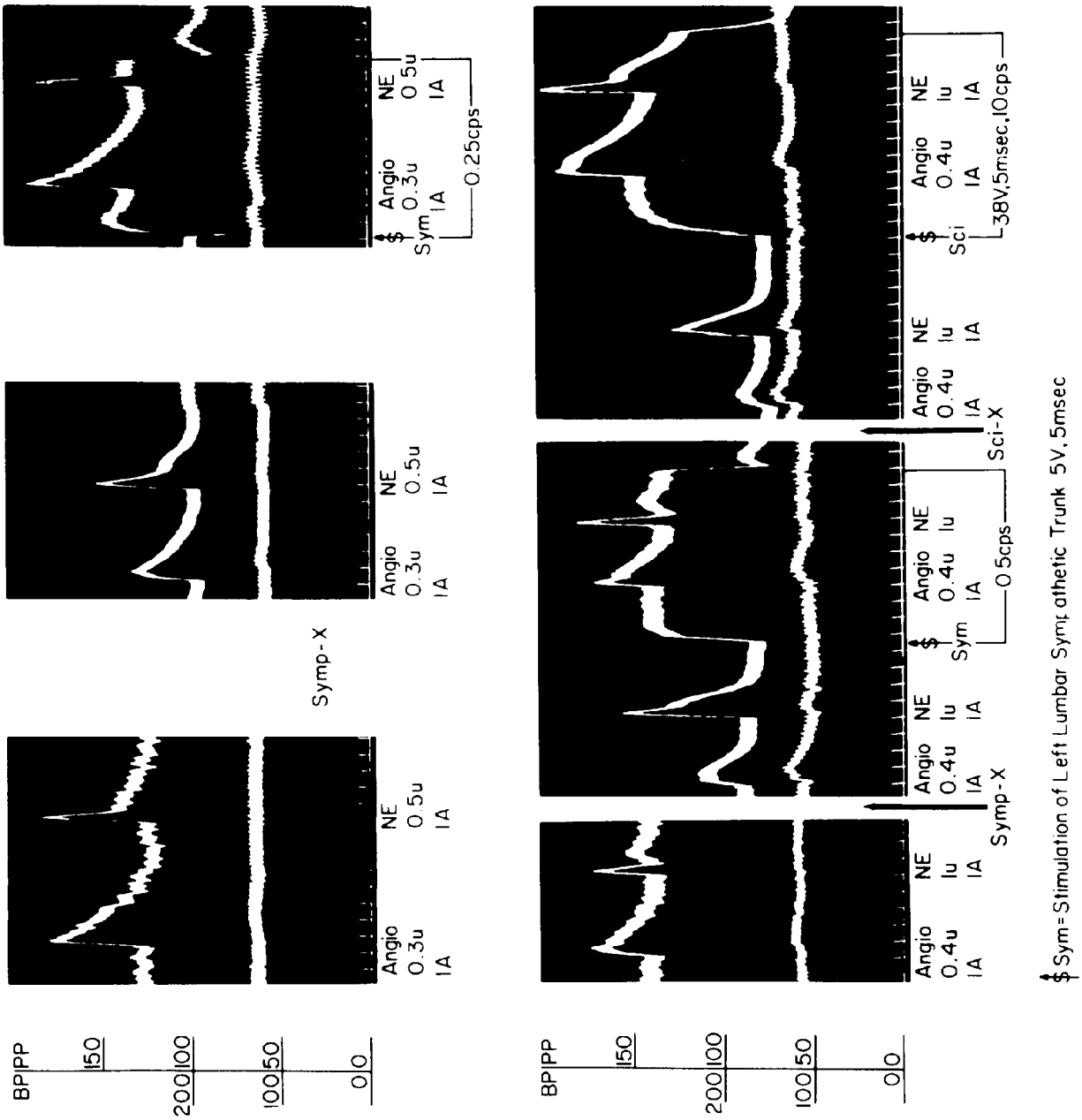

BP
P
P
150
200
100
100
50
0
0
Angio
0.3u
IA
NE
0.5u
IA
Symp-X
Angio
0.3u
IA
NE
0.5u
IA
Angio
0.3u
IA
NE
0.5u
IA
Sym
0.25cps
Angio
0.4u
IA
NE
1u
IA
Angio
0.4u
IA
NE
1u
IA
Sym
0.5cps
Symp-X
Angio
0.4u
IA
NE
1u
IA
Sci
38V,5msec,10cps
Sci-X
$ Sym=Stimulation of Left Lumbar Sympathetic Trunk 5V,5msec

FIGURE 7

The effect of acutely sectioning the sympathetic nerves on the resistance of the hindlimb vasculature and on the vasopressor action of angiotensin for the 29 experiments is illustrated in Figure 8. The per cent reduction of the angiotensin response, plotted on the ordinate, was corrected in all cases for changes in the response to norepinephrine by subtracting from 100 the per cent change in the ratio of angiotensin/norepinephrine. The per cent reduction in perfusion pressure, plotted on the abcissa, was calculated after the perfusion pressure had reached a stable level following decentralization. The slope of the calculated regression line, $Y=1.184X + 6.41$, is significantly different from zero ($p<.001$). Although the point of intercept of the regression line does not pass through zero, it is not significantly different from zero ($p<.5$).

In 5 experiments administration of the ganglion blocking agent, hexamethonium, produced a reduction of the angiotensin response comparable to that seen after decentralizing the sympathetic supply to the hindlimbs.

2. The ability of pre and postganglionic sympathetic nerve stimulation to restore the vasoconstrictor action of angiotensin following acute decentralization.

The third panel of the upper record illustrated in Figure 7 demonstrates that the vasoconstrictor action of angiotensin can be restored to nearly the same magnitude as the control response shown in the first panel, by electrically stimulating the peripheral end of the decentralized lumbar sympathetic trunks. The response to norepinephrine again remained unchanged. A total of 26 experiments were performed

FIGURE 8

CORRELATION OF NEUROGENIC
TONE IN THE PERFUSED EXTREMITIES
AND THE VASOCONSTRICTOR ACTION OF ANGIOTENSIN

Each point represents the change in the angiotensin pressor response and the change in perfusion pressure observed in a single experiment following acute decentralization of the lumbar sympathetic trunks.

The slope of the regression line is described by the equation $Y = 1.184X + 6.41$

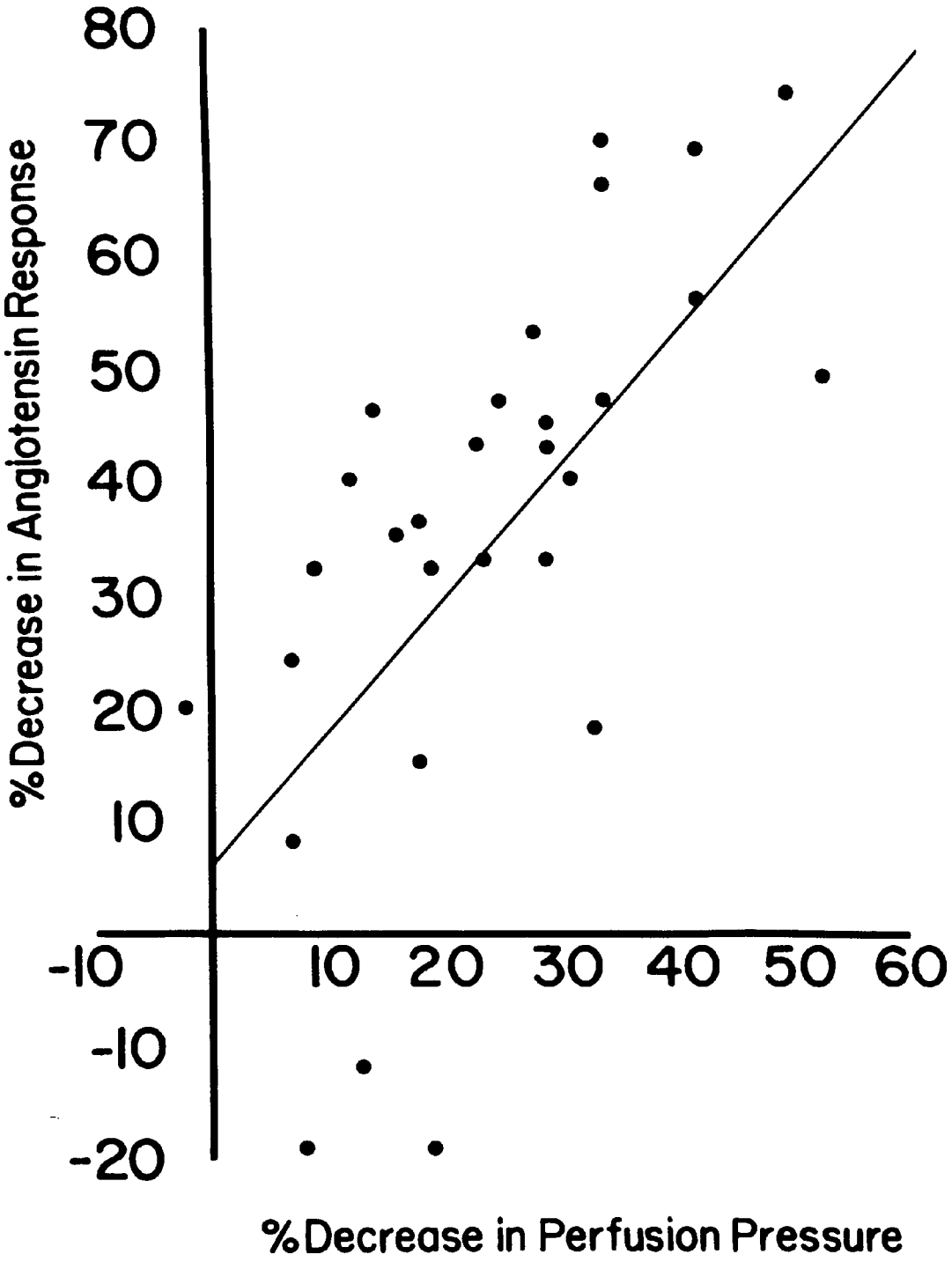

80
70
60
50
40
30
20
10
-10
-20
10 20 30 40 50 60
-10
% Decrease in Angiotensin Response
% Decrease in Perfusion Pressure

FIGURE 8

in which the response to angiotensin was examined at a time when adrenergic tone was being replaced by electrical stimulation of the sympathetic trunks. In these 26 experiments the absolute angiotensin response in mm of Hg was restored to 92 per cent of the control response. If, the change in norepinephrine was taken into consideration, the angiotensin response observed during electrical replacement of lost tone was then calculated to be 103 per cent of the control response.

The lower record in Figure 7 illustrates an experiment in which only the left hindleg was perfused, and again demonstrates the decreased vasoconstrictor action of angiotensin following sympathetic denervation of the hindlimbs, and restoration of the response by electrical excitation of the sectioned nerves. Between the second and third panels the sciatic nerve to the left leg was exposed, sectioned, and prepared for stimulation. In addition the animal was given 0.75 mg/kg of decamethonium to prevent skeletal muscle activity. The third panel shows that stimulation of the distal end of the cut sciatic nerve at the appropriate parameters to elicit a response in the limb comparable to that obtained by stimulation of the sympathetic trunk, was equally capable of augmenting the vasoconstrictor response produced by angiotensin.

In several experiments it was observed that the angiotensin response appeared to be potentiated to a greater extent by low frequency stimulation of the nerves than it was by high frequency stimulation. Thus 5 experiments were performed in which the response to angiotensin was examined during

stimulation of the lumbar sympathetic nerves at 10 cps or less and 30 cps or greater. The results of these experiments are shown in Table 11. The ratio of angiotensin to norepinephrine was significantly increased ($p<.05$) during the low frequency stimulation as compared to the ratio of the responses following denervation of the limb. The ratio was not significantly increased ($p<.1$) during the high frequency stimulation.

Qualitatively the same results were obtained if the absolute pressor responses produced by angiotensin were considered rather than the ratios of angiotensin to norepinephrine. Under these circumstances, again the angiotensin response was significantly increased ($p<.01$) during the low frequency stimulation but not during the high frequency stimulation ($p<.6$).

3. Effect of spinal anesthesia.

Four experiments were performed to test the effect of spinal anesthesia on the potentiation of the angiotensin response by lumbar sympathetic nerve stimulation. In all four experiments the potentiation was as great or greater following spinal anesthesia than prior to it (Table 12A). The degree of vasoconstriction produced by electrical excitation of the sympathetic trunks was, however, usually somewhat increased following spinal anesthesia.

4. Effect of JB-516, guanethidine, reserpine, and Su-4029.

Three experiments were conducted in which JB-516 (Catron), a monoamine oxidase inhibitor, was administered intravenously at a dose of 15 mg/kg. The augmentation of the angiotensin response by sympathetic nerve stimulation was unaltered following this agent (Table 12B). It was noted,

TABLE 11

EFFECT OF STIMULATION FREQUENCY ON
RESTORATION OF ANGIOTENSIN RESPONSE

	Ratios angiotensin/norepinephrine		
Exp. No.	Denervated	Low Frequency	High Frequency
1	0.29	0.76 (2)	0.32 (30)
2	0.65	1.12 (1)	1.25 (30)
3	0.81	1.43 (10)	0.81 (60)
4	0.65	1.93 (1)	1.79 (30)
5	0.76	1.61 (5)	1.21 (30)
Means ±S.E.	0.63 ± 0.203	1.37 ± 0.245*	1.076 ± 0.245**

* Significantly greater than denervated $p<.05$
** Not significantly different than denervated $p<.1$
Lumbar sympathetic trunks stimulated at 5V, 5msec., and the frequency in cycles/sec. indicated in parentheses

TABLE 12

EFFECT OF PHARMACOLOGIC AGENTS ON
POTENTIATION OF THE ANGIOTENSIN RESPONSE
BY LUMBAR SYMPATHETIC NERVE STIMULATION

* - Both agents administered intra-arterially into the perfused limbs. Angiotensin 0.3 mcg total dose; Norepinephrine 0.5 mcg total dose.

non-stim. - Responses recorded following decentralization of the lumbar sympathetic nerves.

stim. - Responses recorded during replacement of lost tone by electrical stimulation of sympathetic nerves at 5V, 5msec, 1cps.

Exp. No.	Ratios angiotensin/norepinephrine* Before non-stim.	Before stim.	After non-stim.	After stim.
	A. Spinal Anesthesia			
1	.46	1.22	.4	1.21
2	.77	1.0	.3	.89
3	.5	1.25	.58	1.5
4	.73	1.0	.6	.91
Average	.62	1.12	.47	1.13
	B. JB-516 (15 mg/kg)			
1	.15	.49	.28	.63
2	.54	.63	.70	1.06
3	.54		1.64	
	C. Su-4029 (20 mg/kg)			
1	.77		.64	1.18
2	.31	.67	.28	.45
3	.24	.57	.25	.48
	D. Reserpine (1 mg/kg)			
1	.69	1.83	.64	1.13
2	.89	1.04	.87	1.17
3	1.05	1.73	.80	.94
4	.67	.95	.33	.85
5	.85	1.63	.73	.97
Average	.83	1.44	.67	1.01
	E. Cocaine (2 mg/kg)			
1	.44	.82	.47	.46
2	.53	.86	.58	.66
3	.65	1.11	.60	.85
4	.57	.92	.81	.98
5	.35	.72	.61	.71
Average	.51	.89	.61	.73

TABLE 12

however, that immediately subsequent to the administration of JB-516 a large increase in resistance, indicated by a rise in perfusion pressure, occurred in the denervated extremities. Following the increased perfusion pressure angiotensin, as compared to norepinephrine, was markedly potentiated. This is shown in Table 12B by the increased ratio of angiotensin/norepinephrine in the non-stimulated state after administration of the agent as compared to before.

In view of the preceding observation with JB-516, guanethidine was administered intravenously to two dogs at a dose of 2 and 5 mg/kg. This agent also provokes an immediate increase in peripheral resistance, and initiated a potentiation of angiotensin in the denervated, nonstimulated state comparable to that observed following JB-516. The potentiated angiotensin response following guanethidine gradually returned to control levels over a 2 hour period, in spite of the fact that the perfusion pressure returned to control levels within about ½ hour. As expected, subsequent to the guanethidine administration stimulation of the sympathetic nerves was ineffective, and the response to angiotensin was unaltered during the nerve stimulation. In one of the two animals JB-516 was administered following guanethidine, and initiated only a slight increase in perfusion pressure.

In 3 experiments Su-4029, hexahydro-1-azepine-propionanidoxime·hydrochloride, an agent possessing pharmacological properties similar to reserpine, evoked only a very slight increase in perfusion pressure, and failed to have an effect on the vasoconstricting action of angiotensin (Table 12C).

The potentiating effect of adrenergic activation on the response to angiotensin was also unaltered following this agent.

Reserpine acutely administered intravenously to 4 dogs at a dose of 1 mg/kg, slightly reduced the ability of preganglionic lumbar sympathetic stimulation to augment the vasoconstrictor action of angiotensin (Table 12D). However, it should also be pointed out that reserpine interfered with the ability of sympathetic nerve stimulation to induce vasoconstriction in the perfused extremities. Furthermore, the per cent decrease in the response to nerve stimulation was determined to correlate very closely to the per cent reduction of the angiotensin/norepinephrine ratio.

5. Effect of cocaine.

Cocaine was administered intravenously at a dose of 2 mg/kg to 5 animals. One of the 5 experiments is depicted in Figure 9. The second panel illustrates the augmentation of the angiotensin response observed during lumbar sympathetic nerve stimulation as compared to the response in the denervated limb shown in the first panel. Between the second and third panels cocaine was administered. In the third and fourth panels the responses to angiotensin and norepinephrine were repeated in the absence and in the presence of sympathetic nerve stimulation. It can be seen that cocaine nearly abolished the potentiating effect of nerve stimulation on the angiotensin response in this experiment as it did in the remaining four (Table 12E). In three of the five experiments the vasoconstrictor tone in the hindlimbs was replaced by stimulation of the lumbar trunks, and in two by stimulation of the distal end of the divided sciatic nerve.

FIGURE 9

EFFECT OF COCAINE ON RESTORATION OF THE ANGIOTENSIN RESPONSE BY SYMPATHETIC NERVE STIMULATION

20.4 kg male dog. Perfusion of the hindquarters. Top tracing: perfusion pressure. Bottom tracing: systemic blood pressure. Scale at the left measures systemic blood pressure (BP) and perfusion pressure (PP) in mm of Hg. Time base: 1 minute

Angio - Angiotensin II amide (Hypertensin, Ciba)

NE - Norepinephrine

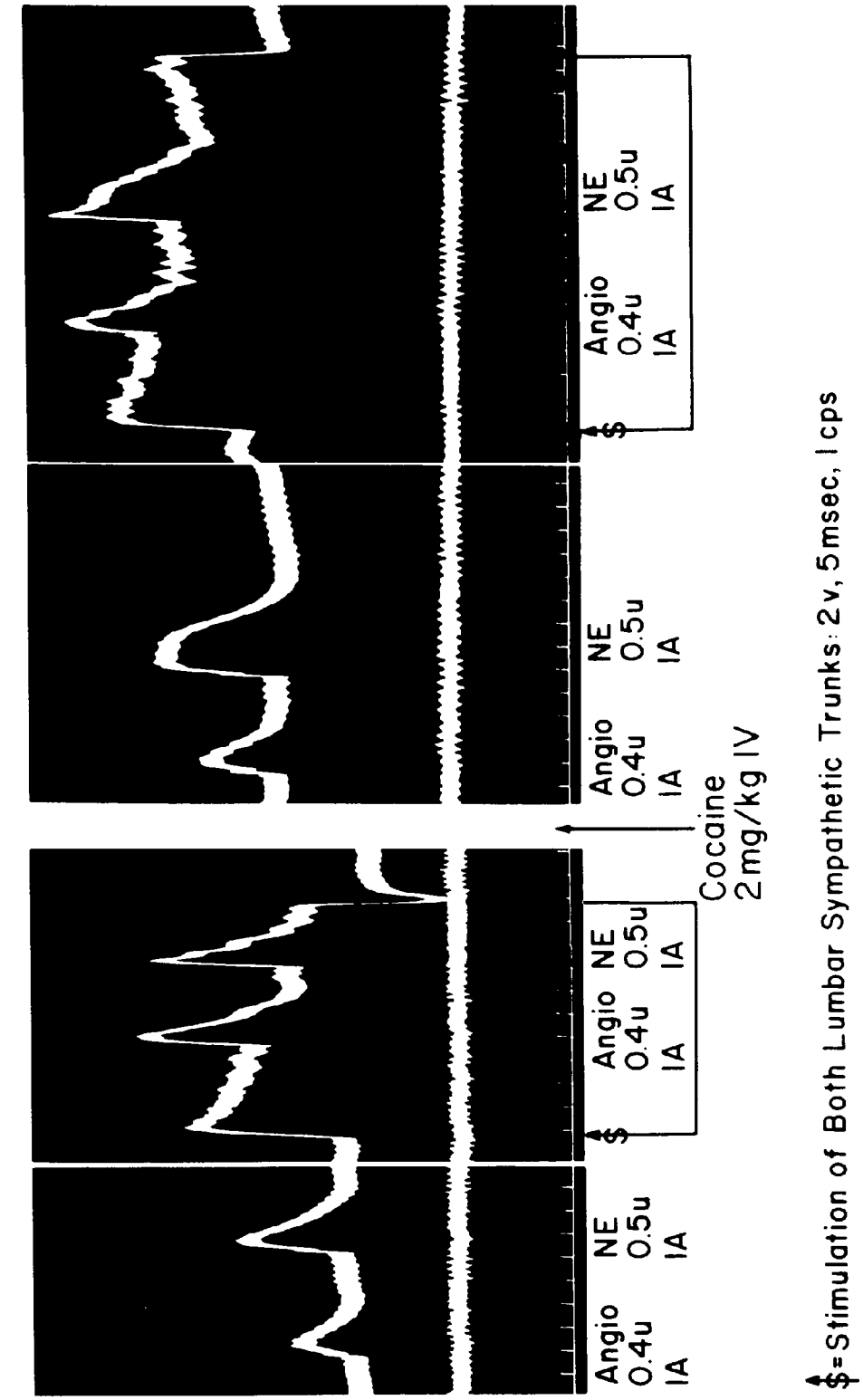

BP IPP
200
150
200 100
100 50
0 0
Angio 0.4u IA
NE 0.5u IA
$
Cocaine 2mg/kg IV
$=Stimulation of Both Lumbar Sympathetic Trunks: 2v, 5msec, 1cps

FIGURE 9

CHAPTER IV

DISCUSSION

A. The importance of the renal pressor system as a compensatory mechanism in acute hypotension

Sapirstein et al. (1941) suggested that the renal pressor system might exert a homeostatic role in the maintenance of arterial blood pressure. This postulate was based solely upon the observation that the blood levels of angiotensin were elevated following reduction of the systemic blood pressure by hemorrhage. No attempt was made by these investigators, however, to prove experimentally the extent to which the renal pressor system functions in the regulation of blood pressure. Many investigators (see INTRODUCTION) have confirmed the observation that renin levels in the blood increase following hypotension, yet Hamilton and Collins (1941) appear to have been the only investigators to attempt to evaluate the practical significance of the increased concentrations of renal pressor material. They reported that upon removal of 38 cc of blood per kilogram of body weight over a 50 minute period, the blood pressure of non-nephrectomized dogs fell to 63 mm of Hg while that of nephrectomized animals fell to 23 mm of Hg indicating that the kidneys were, indeed, playing a role in the compensatory reaction which occurred in response to hemorrhage.

The results of the present investigation indicate that the kidneys do not contribute to the maintenance of vascular tone during the normotensive state in the untreated dog. This argument is based upon the premise that the vascular constricting

action of previously secreted renin would persist for several minutes. Thus if renin were secreted before the assumption of the hypotensive state in normal animals a greater initial blood loss than in nephrectomized animals would be anticipated. Since there was no difference in the amount of blood lost by normal and nephrectomized dogs earlier than two minutes, it must be assumed that the kidney does not contribute to the initial blood loss, and by corollary, that the kidney does not contribute to the maintenance of vascular tone in the normotensive state. The absence of any apparent difference can be seen by examination of Figure 3.

There is ample evidence, however, that the kidney does contribute to the amount of blood lost after the hypotensive level is reached. This is readily apparent from examination of the differences in bleeding volumes between sham-operated and nephrectomized dogs as shown in Figure 3 and 4. The former figure shows the total accumulated blood loss at any given time during the hypotensive period, while the latter figure shows only the additional amount of blood loss starting two minutes after the beginning of rapid hemorrhage. Thus the values shown in Figure 4 show the secondary blood loss at any given time throughout the hypotensive period. The difference in the amount of secondary blood loss has been evaluated statistically as shown in Table 2 and demonstrates that a significant difference is apparent as early as 5 minutes after the onset of hypotension. The difference remains significant until the end of the second hour of controlled hypotension.

It can be concluded from these data that the kidney

contributes greatly to the slow but very considerable blood loss that occurs when a fixed level of severe hypotension is maintained.

The difference in secondary blood loss continues to increase during the first hour of hypotension and then the difference decreases in magnitude. This observation is interpreted to mean that renin continues to be secreted at least during the first hour of hypotension. From that point on there is either a lessening in the amount of renin secreted, a decrease in the amount of angiotensinogen available, or a decrease in the vascular responsiveness to the formed angiotensin.

Other observations (Sapirstein et al., 1942; Hamilton and Collins, 1942; Dexter et al., 1943, Collins and Hamilton, 1944.) suggest that the second of these possibilities is the most likely. Furthermore, in the present investigation we observed that when angiotensin was injected during hypotension, the amount of further blood loss appeared to be just as great at the end of the second as at the end of the first hour. Thus it is unlikely that the vascular responsiveness to the formed angiotensin was altered during the hypotensive period.

A series of 14 experiments was performed in which the bleeding volumes of sham-operated and nephrectomized dogs subjected to arterial pressures of 80 and 60 mm of Hg were examined. Essentially no difference in the bleeding volume occurred at any time between the 4 sham-operated and 2 nephrectomized dogs stabilized at 80 mm of Hg, or between the 4 sham-operated and 4 nephrectomized animals stabilized at 60 mm of Hg. Thus it would appear on the basis of these preliminary

experiments, that a rather severe degree of hypotension is required before the renal pressor system is triggered sufficiently to exert a significant effect upon vascular capacity.

These results are particularly interesting in view of the reports of Mikasa and Mason (1961), Vander (1964) and Skinner et al. (1964). Mikasa and Mason reported a tonic release of renal pressor material from normal kidneys grafted into 24 hour nephrectomized rats, while Vander and Skinner et al. both reported that significant increases of renin levels were demonstrable in blood taken from the renal vein following very slight decreases of renal perfusion pressure. In the light of our results it would seem necessary to conclude that the demonstrable increase in renin secretion observed with slight decreases in renal perfusion pressure is not great enough to exert a significant effect upon vascular capacity. Perhaps the differences in the results obtained with the two assay procedures indicate that the renal pressor system subserves two distinctly separate physiological purposes. A great deal of evidence has been advanced which implicates the renin-angiotensin system in the regulation of aldosterone secretion by the adrenal cortex (see INTRODUCTION), and it may be toward this end that the renin-angiotensin system is activated by relatively small decreases in renal perfusion pressure. With more drastic reductions of renal perfusion pressure, however, sufficient renin may be secreted to exert a significant effect on vascular capacity.

The absence of a difference in bleeding volumes between the sham-operated and nephrectomized dogs stabilized at

pressures above 45 mm of Hg is also puzzling in view of the report by Bunag et al. (1964), who demonstrated an increased release of renal pressor material in dogs subjected to a slow bleeding procedure which did not cause a lowering of the renal perfusion pressure. They reported, in addition, that the ganglion blocking agent, tetraethylammonium chloride, or local anesthetics applied to the renal hilus would block the renin release induced by this procedure, and therefore, assumed that the increased sympathetic activity due to the slow hemorrhage was responsible for stimulating the secretion of renin. In the present experiments maximal reflex activation of the sympathetic nervous system should certainly have occurred at an arterial pressure of 60 mm of Hg if not at 80 mm of Hg, and yet there was no apparent contribution by the kidney to the bleeding volume. On the other hand, it is evident from the large bleeding volumes obtained at these pressures that the sympathetic nervous system was strongly activated. These results, therefore, question whether sympathetic discharge promotes the release of an amount of renin capable of modifying vascular capacity.

In this connection, it should be pointed out that several investigators have reported, as noted in the INTRODUCTION, that angiotensin itself has little or no capacity to directly induce venoconstriction. Accordingly, one would not expect the renal pressor system to exert much of an effect upon the capacity of the total vascular system, because by far the greater volume of blood is contained within the veins. Not withstanding these reports, it is apparent from the RESULTS

that the kidney is, indeed, capable of secreting a substance which did reduce the capacity of the vascular system by a substantial amount. It was for this reason that angiotensin itself was tested for its ability to effect a negative capacity action. With the method used in this investigation it was clearly demonstrated in many experiments by administering a given amount of the polypeptide intravenously, and observing the amount of blood which appeared in the reservoir in response to the injection that angiotensin could indeed exert a negative capacity effect. In fact, the negative capacity effect evoked by angiotensin in these experiments was equal to, or greater than, the negative capacity response produced by an equiweight injection of norepinephrine or epinephrine. Thus in close-chest dogs maintained at a fixed hypotensive pressure it must be unequivocably accepted that synthetic angiotensin is able to decrease vascular capacity. Consequently, the observed difference in bleeding volumes between sham-operated and nephrectomized dogs may justifiably be attributed to the renin-angiotensin system.

If the statement that angiotensin has no direct constrictor effect upon the capacity vessels is true, it becomes necessary to explain how angiotensin can exert a negative capacity effect in the whole animal. First, however, it is perhaps worthwhile to note that reports from various investigators using essentially the same techniques yield conflicting results as to the direct effect of angiotensin on the venous segments. For example, Haddy et al. (1962) reported that angiotensin had no effect on the resistance to flow in the

venous segments, while Emerson et al. (1965) using similar methods observed that on an equimolar basis angiotensin exerted a greater constriction of veins than did norepinephrine in the perfused forelimb. Thus it would appear that further investigation is imperative before one can state with certainty that angiotensin does not possess the capacity to evoke directly venoconstriction.

Nevertheless, if one were to assume that angiotensin is incapable of inducing venoconstriction, the results obtained in the present investigation may alternatively be explained by an indirect action of the renal pressor material. If angiotensin were acting solely to produce arteriolar constriction, then under the conditions of arterial pressure stabilization a drop in pressure would occur beyond the point of constriction. This means that the pressure within the capillaries and veins would be reduced. Consequently, the distending pressure within the veins would be decreased allowing the venous segments to partially collapse, and thereby causing a passive movement of blood into the central venous tree. Such a method for indirectly decreasing venous capacity has been described by Mellander (1960). Unfortunately, the method used in the present investigation does not allow one to determine whether the observed decreases in venous capacity were due to an indirect action, a direct action, or a combination of the two.

The fact that nephrectomized dogs also exhibit a secondary blood loss indicates that a factor other than renin contributes to the blood loss after establishment of fixed severe

hypotension. The possibility that this further blood loss in nephrectomized animals might be due to hemodilution caused by fluid entering the vascular compartment from extracellular or cellular spaces was examined by the taking of serial hematocrits. In all cases, the average hematocrit values were observed to increase rather than decrease following the 2 minute determination. One might suspect that hemodilution could still be occurring in spite of the elevation of hematocrit values, if there was a concomitant increase in the circulating red blood cell mass brought about by emptying of reservoirs containing cell rich blood such as the spleen. However, splenic contraction in particular would be expected to occur immediately following the onset of hemorrhage, and therefore, would not likely influence the hematocrit values to any great extent following the early minutes of hypotension. This conclusion is supported by the observation that the hematocrit values of the non-drug treated, control animals rose sharply during the first 5 minutes of hypotension and then stabilized for about one hour after which they again increased. On the other hand, in the animals treated with either spinal anesthesia or phenoxybenzamine, which effectively block splenic contraction, the hematocrit values decreased during the first 5 minutes of hypotension, and then also stabilized for about one hour before showing a tendency to rise. Therefore, it would appear that the major shifts of red cell mass and plasma volume occur fairly rapidly following the onset of hypotension, and thus it is not likely that the observed secondary bleeding volume not attributable to renin secretion

can be accounted for on the basis of hemodilution.

B. The effect of the sympathetic nervous system on vascular capacity in acute hypotension

To evaluate more completely the homeostatic role of the renal pressor system, the contribution of the sympathetic nervous system to the bleeding volume was determined for comparative purposes. The contribution of the sympathetic nervous system was ascertained by examining the differences in bleeding volume which occurred between the animals treated with spinal anesthesia and the control animals. In Figure 3 it can be seen that the bleeding volumes of the spinally anesthetized dogs are markedly reduced compared to those of the control animals. At 30 minutes the average bleeding volume of the nephrectomized animals with spinal anesthesia was 20 ml/kg, while that of the nephrectomized, control animals was 35 ml/kg. The difference between the two (15 ml/kg) is, therefore, the amount of bleeding which is due to compensatory sympathetic vasoconstriction. If, on the other hand, one compares the bleeding volumes of the sham-operated animals with and without spinal anesthesia at 30 minutes, the difference is determined to be 21 ml/kg. Thus it is apparent that the sympathetic nervous system is exerting a greater effect in the sham-operated than in the nephrectomized animals. The significance of this disparity will be discussed later.

That the reduction of bleeding volume observed with spinal anesthesia was, in fact, due to abolition of sympathetic vasoconstrictor discharge was substantiated by the series of

experiments in which the animals were pretreated with the alpha adrenergic blocking agent, phenoxybenzamine. As indicated in the RESULTS, the bleeding volumes of the animals treated with phenoxybenzamine were only slightly greater than those of the animals treated with spinal anesthesia. The difference between the two probably being due to an incomplete blockade of the adrenergic receptors by the dose of phenoxybenzamine used. This conclusion is substantiated by the fact that injected norepinephrine was still capable of evoking a negative capacity effect in the phenoxybenzamine treated animals. The effect was, however, greatly reduced from that observed in animals without alpha adrenergic blockade. It is also possible that the difference may be accounted for on the basis of a difference in total blood volume of the animals in the two groups, since Nickerson (1962) has reported that within one hour after administration of phenoxybenzamine the plasma volume may increase by as much as 20 per cent. Since blood volume determinations were not performed in the present investigation it is not possible to critically assess this possibility.

In an attempt to further define the effects of the sympathetic nervous system on the bleeding volume, the series of experiments were performed in which the animals were chronically pretreated with large doses of the adrenergic, anti-release and depleting agent, guanethidine. The amount of blood lost by the guanethidine treated dogs was anticipated to be very nearly the same as that observed in the animals treated with spinal anesthesia or phenoxybenzamine, since the large

doses of guanethidine employed in the present experiments have been shown to abolish completely the vasoconstriction induced in the perfused hindlimb of the dog by electrical stimulation of the lumbar sympathetic chains (Beck, 1965). Thus we assumed that compensatory arteriolar and venoconstriction should also have been virtually absent in the nephrectomized dogs treated with guanethidine. Paradoxically, however, both the nephrectomized and sham-operated animals pretreated with guanethidine shed an amount of blood which was almost identical to that of the respective control animals with no drug treatment.

To test whether or not the very large blood loss of the guanethidine treated dogs relative to that of the spinal preparations, was due to adrenergic vasoconstrictor discharge, the rapidly acting alpha adrenergic blocking agent, phentolamine, was administered intravenously. Phentolamine caused a comparable quantity of blood to reinfuse in both control, non-treated animals, and in animals treated with guanethidine (Table 8). From this experiment it is inferred that roughly the same degree of neurogenic vasoconstrictor tone is present in both groups of animals.

Since the neurogenic influence to the resistance vessels is abolished in the animals treated with guanethidine, it must be assumed that the phentolamine sensitive blood loss was brought about by constriction of the capacity vessels. If so, guanethidine must be incapable of interfering with this constriction. That the constrictor substance blockable by phentolamine is neurogenically secreted is further supported

by the observation that spinal anesthesia caused a reinfusion of blood equivalent to that seen with phentolamine in the guanethidine treated animals.

The reports of Gaffney, Bryant, and Braunwald (1962) and Mason and Braunwald (1964), however, appear to be at odds with our observations and interpretations regarding the actions of guanethidine. Gaffney et al. used the technique of major vessel occlusion, in which they occluded the descending thoracic aorta and the inferior vena cava at the level of the heart. After allowing sufficient time (15 seconds) for the arterial and venous pressures to equilibrate, they then recorded the changes in vena caval pressure evoked by bilateral occlusion of the common carotid arteries or by electrical stimulation of the central end of the sectioned vagus nerve. In this preparation both guanethidine and reserpine were reported to completely abolish the increased venous pressure observed in response to either carotid occlusion or central vagal stimulation. In the latter paper, Mason and Braunwald reported that reserpine and guanethidine abolished the reflex venoconstriction which occurred in the human forearm in response to leg exercise or to immersion of the opposite hand in ice water.

In support of our observations are the findings of Stone et al. (1963), who reported that in dogs chronically treated with reserpine the catecholamine content of the inferior vena cava and the jugular veins were not significantly lowered, whereas the catecholamine content of the aorta and the carotid arteries was reduced significantly. Although they

did not examine the effect of guanethidine on catecholamine levels, their data, nevertheless, tend to indicate that the adrenergic depleting or antirelease agents may exert a selective action upon resistance and capacitance vessels.

Other evidence that reserpine may selectively deplete arterial catecholamines without affecting venous catecholamines is provided by the work of Abboud and Eckstein (1964). They found that supersensitivity to norepinephrine developed in the reserpinized dog only with respect to the response of the arterioles. They interpreted this to indicate that the norepinephrine stores in the veins were negligible in the control state, and thus reserpine could have little effect. Alternatively, their results could be explained by positing that reserpine failed to deplete the veins of catecholamines. Such an explanation is favored by the report of Reynolds and Imig (1963), who showed that the venous response to norepinephrine was significantly augmented following chronic, surgical sympathectomy.

If the antirelease and/or depleting agents do, in fact, exert a selective action on the catecholamine stores of the various vascular segments it must indicate that some anatomical or functional difference exists between the innervation of the arterial vessels and the venous vessels. These results may, therefore, open up a new dimension in autonomic control of the vascular system.

C. <u>The contribution of the heart to the bleeding volume</u>

It can be seen by comparing Figure 3 and Figure 5 that during the early minutes of hemorrhage the rate of bleeding

was considerably faster in the animals treated with phenoxybenzamine than it was in the animals treated with the spinal anesthesic. This is evidenced by the finding that the nephrectomized dogs of the phenoxybenzamine-treated group reached a plateau in the bleeding curve at about 6 minutes, whereas the nephrectomized animals under spinal anesthesia did not reach a similar plateau until about 14 minutes after the onset of hypotension. The observed difference in the initial rate of hemorrhage is most logically explained by the fact that phenoxybenzamine does not block the effects of the sympathetic nervous system on the heart, whereas spinal anesthesia does. Therefore, the animals treated with phenoxybenzamine maintain the capacity to respond to hemorrhage by increasing both the rate and the force of contraction of the heart. This explanation is further substantiated by a comparison of the initial hemorrhage rates of the control animals and the guanethidine treated animals, which show a difference similar to that noted between the animals treated with spinal anesthesia and those with phenoxybenzamine. The control nephrectomized animals reached the plateau in the bleeding curve at 6-7 minutes as did the phenoxybenzamine treated animals, while the nephrectomized animals with guanethidine, like those with spinal anesthesia, reached the plateau at 12-14 minutes. In view of the fact that guanethidine also effectively abolishes the sympathetic influence on the heart, and caused a reduction in the initial rate of hemorrhage similar to that seen with spinal anesthesia, it was concluded that reflex sympathetic activation of the heart

definitely played a role in the initial rate of bleeding.

The effect of increased sympathetic activity to the heart on the volume of blood accumulating in the reservoir over a longer period of time, however, is not so clear. One might suspect that the slightly greater bleeding volumes of the phenoxybenzamine treated animals as compared to the animals with spinal anesthesia is at least, partially due to the reflex activation of the heart in the phenoxybenzamine animals. However, in 5 of the phenoxybenzamine treated animals the beta adrenergic blocking agent, 1-isopropylamino-3-(1-napthyl oxy)-2-propanol hydrochloride (Inderal), was given intravenously 2 hours after the onset of hypotension, and little or no reinfusion of blood occurred. Therefore, it is highly unlikely that the increased sympathetic activity to the heart was contributing to the accumulation of blood in the reservoir over a longer period of time. A similar conclusion must be reached from the observation that the bleeding volumes of the guanethidine treated nephrectomized animals are not different from those of the control nephrectomized animals after 15 minutes of hypotension.

As shown in Table 7, however, it was noted that spinal anesthesia administered 1 hour after the onset of hypotension caused a significantly greater reinfusion of blood in control animals than it did in animals which had been pretreated with guanethidine. Furthermore, this additional reinfusion with spinal anesthesia in the control animals could be demonstrated following the prior administration of phentolamine. Thus it was suspected that the additional reinfusion was due to

blockade of the sympathetic innervation of the heart. To test this hypothesis Inderal was slowly administered intravenously at a dose of 2 mg/kg, in place of spinal anesthesia, after phentolamine in animals with and without guanethidine treatment. In those animals treated with guanethidine Inderal was found to have only a minimal effect on the volume of blood in the reservoir, however, in the control group the animals died within 3-4 minutes after beginning the administration of the beta blocking agent. The death appeared to be due to cardiac arrest, and thus raises an interesting question as to the mechanism involved, but unfortunately does not provide the information for which the experiment was intended.

Thus the increased sympathetic activity to the heart undoubtedly shortens the time required for the animal to reach the fixed hypotensive level. However, once the fixed hypotensive level is reached the cardiac output is in all probability so severely limited by the venous return that sympathetic drive to the heart is no longer effective in so far as being able to influence the bleeding volume.

D. The existence of an interaction between the sympathetic nervous system and the renal pressor system

That a portion of the vasoconstrictor action of angiotensin is attributable to an interaction between the polypeptide and the sympathetic neurons has been described by several investigators as reviewed in the INTRODUCTION. The physiological importance of this interaction has, however, not been demonstrated. The present investigation not only demonstrates that such an interaction does occur in the hypotensive animal, but

shows in addition that it is responsible for approximately 17 per cent of the bleeding volume which is observed in response to acute hemorrhage.

The 17 per cent value for the interaction component was obtained by determining the difference in bleeding volumes at 30 minutes between the nephrectomized dogs with and without spinal anesthesia (15 ml/kg), and the difference between the sham-operated dogs with and without spinal anesthesia (22 ml/kg). These differences are equivalent to 33 per cent and 50 per cent respectively of the bleeding volume of the control, sham-operated animals. The difference between the two thus being an index of the interaction of the renal pressor system with the sympathetic nervous system. It is important to note that the above determinations were based upon the bleeding volumes recorded 30 minutes after the onset of hypotension. The 30 minute recordings were chosen for this analysis because at this time the maximum effect of the renal pressor system was manifest. Therefore, the above value for the contribution of the interaction to the bleeding volume is the maximum value which could be obtained with the present experimental conditions.

If, on the other hand, one compares the renal contribution to the bleeding volume of the animals with and without spinal anesthesia the evidence for an interaction between the two systems is even more striking. In fact, the difference between the control shammed and nephrectomized animals is somewhat more than twice the difference between the shammed and nephrectomized animals with spinal anesthesia.

The component of interaction between the two systems was further demonstrated and validated statistically by using the secondary bleeding volumes of the control animals and the animals with spinal anesthesia. The two minute recording was chosen as the initial bleeding volume, and thus the secondary bleeding volume is the difference between the initial bleeding volume and the volume recorded at subsequent times. The two minute volume was selected as the initial bleeding volume primarily because there was essentially no difference between the sham-operated and the nephrectomized animals at this time, thus indicating that the renal pressor system had not yet begun to exert an effect upon the bleeding volume. Within the next minute however, the sham-operated animals began to show a greater bleeding volume per unit of time than did the nephrectomized animals, indicating that the renal pressor system was beginning to influence the bleeding volume within 3 minutes after the onset of hypotension. The time required for the renal pressor system to exert an effect in the present investigation agrees reasonably well with the observation made by Skinner et al. (1964), who stated that increased levels of renin could be detected in the renal venous blood within 1½ minutes after decreasing the renal perfusion pressure by aortic constriction. The secondary bleeding volumes were utilized for this analysis in preference to the total accumulated blood volumes primarily because there was much less animal to animal variation in the former than the latter. This is shown by the considerably smaller standard errors calculated for the mean secondary bleeding volumes than

those calculated for the total bleeding volumes. Thus, the large animal to animal variation in bleeding volume occurs primarily with respect to the initial bleeding volume, and is undoubtedly due to such things as differences in total blood volume, health of the animals, environmental conditions etc.

The effect of the sympathetic nervous system on the renal pressor system was determined by analysing the differences in secondary bleeding volumes which occurred between the sham-operated and nephrectomized animals with spinal anesthesia and those without spinal anesthesia. The difference which occurred in the animals with spinal anesthesia was only 35 per cent of the difference which occurred between the animals without spinal anesthesia. Stated in another way, the contribution of the kidney to the secondary bleeding volume is reduced 65 per cent by the administration of spinal anesthesia. Thus under the conditions of this investigation only 35 per cent of the renal contribution to the secondary bleeding volume can be attributed to a direct vasoconstricting action of the renal pressor material, whereas 65 per cent of the renal contribution must be attributed to an interaction between the renal pressor material and the sympathetic nervous system.

That the observed interaction does, in fact, involve the sympathetic nervous system and not some other system also amenable to blockade by spinal anesthesia, is evidenced by the fact that the renal contribution to the bleeding volume was also reduced in the animals treated with phenoxybenzamine. In the animals treated with guanethidine, however, again the

paradoxical observation was made that guanethidine, unlike spinal anesthesia or phenoxybenzamine, did not affect the interaction between the two systems. In accord with the hypothesis presented previously that the bleeding volume is determined primarily by venoconstriction and that guanethidine is incapable of preventing this venoconstriction, one must now postulate that the interaction between the adrenergic nervous system and the renal pressor system can also occur with respect to the venous innervation.

E. Possible sites and mechanisms for the interaction between the sympathetic nervous system and the renal pressor system

The exact site at which, and the mechanism by which the sympathetic nervous system is able to interact with the renal pressor system is as yet uncertain. There would appear to be at least two sites at which such an interaction could conceivably occur. First, the increased sympathetic activity induced by hemorrhage may act directly upon the kidney to augment the release of the renal pressor substance. As was discussed earlier, Bunag et al. (1964) have demonstrated that intense sympathetic activity to the kidney can, in fact, promote the release of renin. Although we were unable to demonstrate in the present investigation, a significant effect of the renal pressor system on vascular capacity in those animals stabilized at an arterial pressure of 60 mm of Hg when maximal sympathetic activation would be expected, it remains conceivable that the increased sympathetic activity may augment the release of renin brought about by a further decrease in renal perfusion pressure. However, if this were true one would

expect that guanethidine, as well as spinal anesthesia and phenoxybenzamine, should have reduced the renal component of the bleeding volume. Unless guanethidine is also unable to antagonize the sympathetic innervation of the kidney.

Secondly, the interaction may occur at the peripheral nerve terminals. As mentioned in the INTRODUCTION, Zimmerman (1962) has shown that the vasoconstrictor action of angiotensin in the perfused hind extremities of the dog, is partially dependent upon the sympathetic tone to the extremities. In addition, McCubbin and Page (1963) have demonstrated that the response evoked by occlusion of the common carotid arteries on the perfused mesenteric artery preparation was enhanced during an infusion of angiotensin. Electrical activity recorded from the splanchnic nerve during carotid occlusion, however, was not significantly different in the presence of or absence of angiotensin. Therefore, the authors concluded that the neurogenic effect of angiotensin must be associated with the peripheral adrenergic nerve terminals.

As illustrated in Figure 7 of the RESULTS, we have confirmed the observation made by Zimmerman that the pressor action of angiotensin is reduced following denervation of the perfused hind extremities. In 29 experiments the angiotensin response was found to be reduced by an average of 44 per cent. The per cent reduction of the response was proven to be related to the amount of sympathetic tone present prior to denervation as shown by the significance of the slope of the calculated regression line (Figure 8 in the RESULTS). Furthermore, in 26 experiments it was demonstrated that the average

pressor response produced by angiotensin could be restored to 92 per cent of the control response when the lost adrenergic tone was replaced by electrically stimulating the lumbar sympathetic trunks. Zimmerman also reported that the response to angiotensin could be augmented by electrically stimulating the sympathetic nerves, however, in his experiments the response was restored to only 83 per cent of the control response. The primary difference between the experiments reported by Zimmerman and those of the present investigation appears to be the parameters of nerve stimulation required to restore the lost tone. In the former experiments stimulation frequencies of 10-32 cps were utilized while in the present experiments frequencies ranging from 0.1 - 2.0 cps were used. The voltage and duration of the impulses were roughly comparable in both investigations. Whether or not the difference in the frequency of nerve stimulation is the sole explanation for the discrepancy in the results cannot be stated with certainty, however, it is most unlikely that the adrenergic neurons would have been firing at a frequency in excess of 10 cps prior to denervation. Therefore, suggesting that perhaps a portion of the fibers were damaged by the act of denervation, thus requiring that the remaining fibers be stimulated at a higher frequency in order to restore the tone to the same level which existed prior to denervation. It should be pointed out that although the average response to angiotensin was almost completely restored by replacement of tone in the present investigation, the degree of restoration from experiment to experiment was highly variable. This variability is probably due to the fact that it is

extremely difficult to exactly replace the lost tone, and probably also indicates that even when the tone is replaced the neurogenic activity does not precisely duplicate the pre-existing physiological activity.

The interaction between angiotensin and the sympathetic nervous system does not involve the central nervous system or the sympathetic ganglia as evidenced by the following two observations: 1.) anesthesia of the spinal cord has no effect on the capacity of preganglionic sympathetic nerve stimulation to restore the vasopressor action of angiotensin, and 2.) restoration of the response could be effected equally be either preganglionic sympathetic nerve stimulation or stimulation of the postganglionic fibers in the sciatic nerve. Thus, these observations substantiate the conclusion of McCubbin and Page that the interaction must occur in the vicinity of the neuro-effector junction.

Consequently, the problem now becomes one of resolving the mechanism by which angiotensin is capable of rendering a given neurogenic impulse more effective. There appear to be at least three possibilities by which this could occur. 1.) Angiotensin may sensitize the adrenergic receptors in such a way that a given amount of released catecholamine is able to exert a greater effect, or conversely, that the released norepinephrine can increase the sensitivity of the vasculature to the direct vasoconstricting action of angiotensin. 2.) Angiotensin may be capable of facilitating the release of amines from the adrenergic terminals. 3.) It may interfere with the mechanisms responsible for the termination

of the released amines. Since it has been postulated that catecholamines are at least partially terminated by being taken back into the stores from which they were released, it is conceivable that angiotensin is interfering with the mechanisms responsible for this re-uptake. It is also possible that angiotensin may interfere with the action of the enzymes responsible for catecholamine destruction. Both of the aforementioned possibilities for interference with normal processes of catecholamine termination would effectively result in a greater concentration of the adrenergic mediator at the receptor sites.

The authors are not at the present time aware of any evidence which would strongly support the first hypothesis. The observations that the vasoconstrictor action of angiotensin is not potentiated by infusions of norepinephrine, and that norepinephrine is not potentiated by the infusion of angiotensin (Zimmerman 1962, 1964) would, in fact, argue strongly against any receptor sensitization phenomenon. However, the fact remains that during an infusion of angiotensin at a time when the response to exogenously administered norepinephrine is unchanged or slightly decreased, there is an increased response to neurogenically liberated norepinephrine. Therefore, one cannot eliminate the possibility that there may be a difference in the ability of exogenously administered and endogenously released norepinephrine to gain access to the receptor area, or that there may be more than a single receptor involved.

In view of the evidence available it would appear that

the more probable mechanism for the neurogenic activity of angiotensin is concerned with either the mechanisms of release or termination of catecholamines at peripheral adrenergic nerve endings. The observation that the monoamine oxidase inhibitor, JB-516, was unable to alter the increased angiotensin response observed during stimulation of the lumbar sympathetic nerves was accepted as evidence against the postulation that angiotensin might interfere with the alteration of norepinephrine by the enzyme monoamine oxidase. However, the alternate pathway for catecholamine termination through catechol-o-methyl transferase was not investigated, and remains a possibility.

Since it appeared that the two most probable mechanisms for the neurogenic action of angiotensin were either to facilitate the release of, or inhibit the re-uptake of catecholamine, experiments were designed in an attempt to distinguish between these two possibilities. Brown and Gillespie (1957) reported that maximal norepinephrine overflow occurred in the spleen when the splanchnic nerve was stimulated at a frequency of 30 cps. They explained this observation on the basis that at a frequency of 30 cps the tissue receptors become saturated, and thus a large spillover of transmitter occurs. More recently the concept that adrenergic neurons themselves may play an important role in the removal of catecholamines has been introduced (Whitby et al. 1960, 1961; Hertting et al. 1961.). Paton (1960) suggested that the reincorporation of released transmitter may by a physiological process. If this is true one would expect a greater

quantity of transmitter to accumulate at receptor areas at high frequency stimulations than at low frequency stimulations, because the time for re-uptake to occur would be encroached upon by the rate of firing of the nerve.

If re-uptake of catecholamines by nerve endings can be inhibited by high frequency stimulation, it was hypothesized that providing angiotensin were also acting by inhibiting re-uptake one should be able to demonstrate a greater facilitation of the angiotensin response by low frequency stimulation of the sympathetics than by high frequency stimulation. As indicated in the RESULTS, there was a significantly greater increase in the response to angiotensin during low frequency stimulation than during high frequency stimulation in our preparation. Comparable results have been reported by Benelli, G. et al. (1964) using the isolated vas deferens preparation. If, on the other hand, angiotensin were acting to facilitate the release of adrenergic transmitter one would not expect that it would be frequency limited. Therefore, the above evidence would appear to support the postulate that angiotensin is interfering with the mechanisms responsible for re-uptake of released catecholamines.

It should be mentioned that the converse experiments in which the vasoconstrictor responses to various frequencies of sympathetic nerve stimulation were examined before and during an infusion of angiotensin, failed to provide any clear-cut correlation between frequency of stimulation and potentiation of the response. In three of the six experiments performed in this manner the response to low frequency stimulation was

increased to a greater degree than the response to high frequency stimulation. However, in the remaining three experiments there was no increase in the response to stimulation of the sympathetic nerves at any frequency. In fact, in two of the three animals the neurogenic responses were actually decreased during the angiotensin infusion. We do not at the present time have any explanation for this discrepancy between the two groups of seemingly parallel experiments.

In as much as Hertting et al. (1961) have shown that cocaine is able to effectively interfere with the re-uptake of norepinephrine by sympathetically innervated effector organs, it follows that if angiotensin is also exerting its neurogenic effect by inhibiting uptake then cocaine should block the potentiation of angiotensin observed during stimulation of the adrenergic nerves. As shown by the results this hypothesis proved to be most tenable. In the five experiments attempted cocaine nearly abolished the augmentation of the angiotensin pressor response brought about by sympathetic stimulation. This observation was accepted as being highly indicative that angiotensin was functioning neurogenically by interfering with the reincorporation of norepinephrine by the nerve endings. That cocaine is capable of blocking re-uptake by the nerve ending has also been reported by Kirpekar and Cervoni (1963), who showed that cocaine significantly increased norepinephrine output from the spleen at a stimulation frequency of 10 cps but not at a frequency of 30 cps.

The augmentation of angiotensin as compared to

norepinephrine following the large pressor response produced by JB-516 or guanethidine, at a time when no sympathetic nerve activity is present in the hindlimbs, appears to offer no solution to the problem. The pressor response produced by these two agents is undoubtedly due to the release of catecholamines from tissue stores. If re-uptake is a physiological process occurring as a result of nerve activity it is questionable whether or not such a phenomenon would occur in relation to spontaneous discharge of mediator from the stores. However, if re-uptake does, in fact, occur following release by this means, then inhibition of uptake could explain the observed effect of angiotensin. Since it is equally conceivable that under these circumstances angiotensin may be acting to further increase the drug induced release, it is impossible to reach a definite conclusion. That angiotensin itself may, in fact, possess an ability to release catecholamines has been demonstrated by Feldberg and Lewis (1963) using the adrenal medulla.

Reserpine and Su-4029 (a reserpine-like agent) did not produce large increases in the perfusion pressure, and were unable to affect the angiotensin response in the denervated, non-stimulated state. In spite of the fact that reserpine has been reported to inhibit uptake of norepinephrine by sympathetically innervated organs at a dose of 3mg/kg, (Hertting et al. 1961) in the present investigation reserpine, unlike cocaine, did not abolish the augmentation of angiotensin by sympathetic stimulation. However, it did slightly reduce the potentiation of the angiotensin response during

sympathetic stimulation, but the reduction was proportionate to the decreased effect of stimulation seen after reserpine. Consequently, it is doubtful that reserpine had any effect on the action of angiotensin.

At the present time we do not feel that we can state with certainty the exact mechanism underlying the neurogenic activity of angiotensin, since the results obtained in this investigation do not appear to be completely compatible with either the hypothesis that angiotensin is acting to facilitate release of catecholamines from adrenergic nerve endings, or that it is inhibiting re-uptake of the released transmitter. At any rate, the present investigation has further substantiated that the sympathetic nervous system can interact with the renal pressor system at the level of the adrenergic nerve terminals, and possibly also within the kidney. In addition, this interaction has been shown to provide a significant contribution to the compensatory vasoconstriction which occurs in response to hemorrhage.

Figure 10 summarizes the contribution of the various systems to the accumulated blood loss of an intact animal throughout the period of sustained hypotension. The stippled area in the upper portion of the graph represents the contribution of the sympathetic nervous system to the bleeding volume, and the cross-hatched area in the center portion depicts the contribution of the renal pressor system. The area encompassed by the overlap between the stippled area and the cross-hatched area depicts the bleeding volume contributed by the interaction between the sympathetic nervous system and the

FIGURE 10

SCHEMATIC ILLUSTRATION OF THE CONTRIBUTION
OF THE VARIOUS SYSTEMS TO THE BLEEDING VOLUME

See text for description

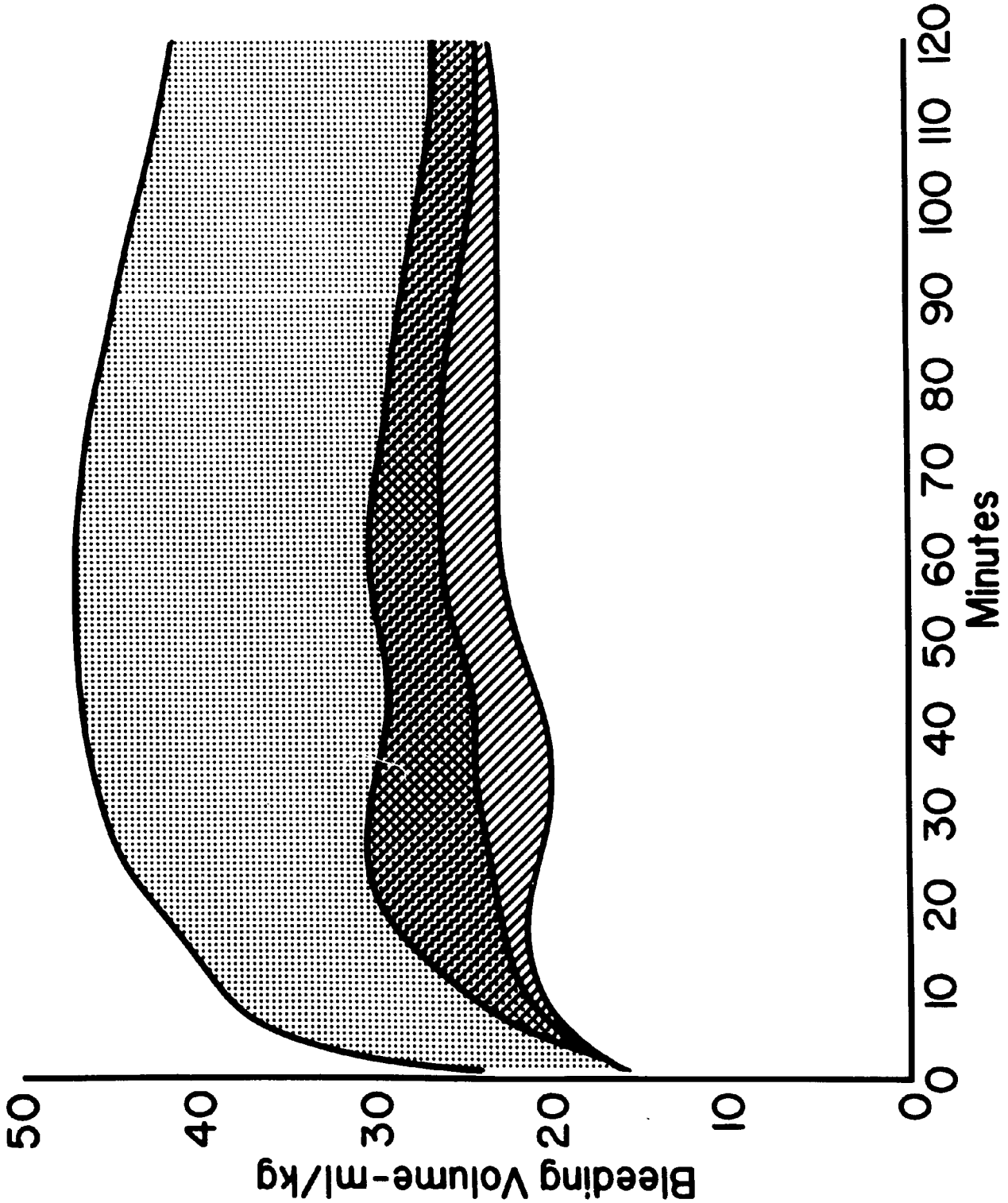

Bleeding Volume-ml/kg
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100 110 120
Minutes

FIGURE 10

renal pressor system. The clear area completing the lower portion of the graph represents the amount of blood lost due to decreased vascular distension brought about by the reduction of arterial pressure, and the contribution of any other, as yet undefined, factors which also may aid in the compensatory vasoconstriction occurring in response to severe hemorrhage.

SUMMARY

The results of the present investigation demonstrate that the renal pressor system definitely contributes to the compensatory vasoconstriction which occurs following a sudden, severe decrease in systemic blood pressure. In fact, following hemorrhage the renal pressor system was determined to contribute up to one-half as much vasoconstriction as did the sympathetic nervous system. In addition, it was clearly established that an interaction occurs between the renal pressor system and the sympathetic nervous system, and that this interaction also accounts for a significant amount of the blood lost during the hypotensive period. The exact site or sites at which the interaction occurs cannot be stated with certainty. However, since the pressor activity of angiotensin in the perfused hindlimb was shown to be partially dependent upon sympathetic tone to the limb, at least a portion of the interaction probably occurs at the sympathetic neuro-effector junction. The mechanism by which the interaction is occurring is also unclear at the present time. In view of the observations that the interaction in the perfused limb was antagonized by cocaine and by high frequency stimulation of the sympathetic nerves, the hypothesis was advanced that angiotensin is capable of interfering with the mechanisms responsible for reincorporation of released mediator by the sympathetic nerve endings. The results of the present investigation do not, however, make it possible to completely rule out the alternative explanation that

angiotensin is acting to facilitate the neurogenic release of catecholamines.

Both spinal anesthesia and phenoxybenzamine markedly decreased the bleeding volume observed in response to sustained hypotension, and also greatly decreased the effect of the renal pressor system. Guanethidine, on the other hand, had virtually no effect on either the observed bleeding volume or the effect of the renal pressor system. This paradoxical observation was interpreted to indicate that guanethidine lacks the capacity to interfere with reflex constriction of the capacity vessels.

BIBLIOGRAPHY

Abboud, F. M. and Eckstein, J. W.: Venous and arterial responses to norepinephrine in dogs treated with reserpine. Am. J. Physiol. 206:299, 1964.

Alexander, R. S.: The participation of the venomotor system in pressor reflexes. Circ. Res. 2:405, 1954.

Barcroft, H. and Swan, H. J. C.: Sympathetic Control of Human Blood Vessels. London: Arnold, 1953.

Bayliss, W. M.: On the physiology of the depressor nerve. J. Physiol., 14:303, 1893.

Beck, L.: An automatic blood pressure stabilizer and volume recorder. Univ. Michigan M. Bull. 24:118, 1958.

Beck, L.: Active reflex dilatation in the innervated perfused hindleg of the dog. Am. J. Physiol. 201:123, 1961.

Beck, L.: The vasoconstrictor capacity of the adrenergic and renopressor systems. Pharmacologist 7:32, 1965.

Beck, L.: Unpublished observations. 1965.

Benelli, G., Della Bella, D. and Gandini, A.: Angiotensin and peripheral sympathetic nerve activity. Brit. J. Pharmacol. 22:211, 1964.

Bernard, C.: Influence de grand sympathique sur la sensibilité et sur la calorification. C. R. Soc. Biol., Paris 3:163, 1851.

Bianchi, A., DeSchaepdryver, A. F., DeVleeschhouwer, G. R. and Preziosi, P.: On the pharmacology of synthetic hypertensin. Arch. int. Pharmacodyn. 124:21, 1960.

Bicherton, R. K. and Buckley, J. P.: Evidence for a central mechanism in angiotensin induced hypertension. Proc. Soc. Exptl. Biol. and Med. 106:834, 1961.

Bing, J. and Kazimierczak, J.: Renin content of different parts of the juxtaglomerular apparatus. Acta. Path. Microbiol. Scand. 54:80, 1962.

Braun-Menendez, E.: Pharmacology of renin and hypertensin. Pharm. Rev. 8:25, 1956.

Brown, G. L. and Gillespie, J. S.: The output of sympathetic transmitter from the spleen of the cat. J. Physiol. 138:81, 1957.

Brown-Sequard and Bernard, C.: (1852) Quoted from: Medical Physiology (P. Bard ed.) C. V. Mosby Co. 1961.

Bumpus, F. M., Schwarz, H. and Page, I. H.: Synthesis and pharmacology of the octapeptide angiotenin. Science 125:886, 1957.

Bunag, R. D., McCubbin, J. W. and Page, I. H.: Neurogenic stimulation of renin release. Pharmacologist 6:201, 1964.

Collins, D. A. and Hamilton, A. S.: Changes in the reninangiotensin system in hemorrhagic shock. Am. J. Physiol. 140:499, 1944.

Cook, W. F. and Pickering, G. W.: Localization of renin within the kidney. J. Physiol. 143:78, 1958.

Cooper, A.: Some experiments and observations on tying the carotid and vertebral arteries, and the pneumo-gastric, phrenic, and sympathetic nerves. Guy's Hosp. Rep., 1:457, 1836.

Cyon, E. De, and Ludwig, C.: Ber. sachs Ges. (Akad.), Wiss., 18:307, 1866.

Davis, J. O.: Angiotensin system and control of aldosterone. In: Angiotensin Systems and Experimental Renal Disease (Metcoff, J., ed.) Proc. 14 annual conf. on the kidney, sponsored by National Kidney Disease Foundation. Little, Brown, and Co. 1963.

Deane, H. W. and Masson, G. M. C.: Adrenal cortical changes in rats with various types of experimental hypertension. J. J. Clin. Endocrinol. 11:193, 1951.

Dexter, L., Frank, H. A., Haynes, F. W. and Altschule, M. D.: Traumatic Shock. VI. The effect of hemorrhagic shock on the concentration of renin and hypertensinogen in the plasma of unanesthetized dogs. J. Clin. Invest. 22:847, 1943.

Divry, A.: The mechanism of the hypertensive action of the kidney. Arch. Internat. Physiol. 59:211, 1951.

Donegan, J. F.: The physiology of the veins. J. Physiol. 55:226, 1921.

DuCharme, D. W., Levin, J. A., and Beck, L.: Relative effects of adrenergic and renopressor systems on vascular capacity during controlled hypotension. Fed. Proc. 24:364, 1965.

Edelman, R. and Hartroft, P. M.: Localization of renin in juxtaglomerular cells of rabbit and dog through use of the fluorescent-antibody technique. Circulation Res. 9:1069, 1961.

Elliot, D. F. and Peart, W. S.: Amino-acid sequence in a hypertensin. Nature 177:527, 1956.

Feldberg, W. and Lewis, G. P.: The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. J. Physiol. (Lond.) 171:98, 1964.

Emerson, T. E. Jr., Hinshaw, C. B., and Brake, C. M.: Vascular effects of angiotensin and norepinephrine in dog, cat, and monkey. Am. J. Physiol. 208:260, 1965.

Finnerty, F. A. Jr.: Hemodynamics of angiotensin in man. Circulation 25:255, 1962.

Fleisch, A.: Venomotorenzentrum and venenreflexe II. Blutdruckzügler und venenreflexe. Pflüger's Arch. 226:393, 1931.

Folkow. B.: Nervous control of the blood vessels. Physiol. Rev. 35:629, 1955.

Folkow, B., Johansson, B. and Mellander, S.: The comparative effects of angiotensin and norepinephrine on consecutive vascular sections. Acta physiol. scand. 53:99, 1961.

Folkow, B., Lewis, D. H., Lundgren, O., Mellander, S., and Wallentin, I.: The effect of graded vasoconstrictor fibre stimulation on the intestinal resistance and capacitance vessels. Acta physiol. scand. 61:445, 1964.

Friedman, B., Abramson, D. I. and Marx, W.: Pressor substance in the cortex of the kidney. Am. J. Physiol. 124:285, 1938.

Gaffney, T. E., Bryant, W. M. and Braunwald, E.: Effects of reserpine and guanethidine on venous reflexes. Circulation Res. 11:889, 1962.

Ganong, W. F., Mulrow, P. J., Boryczka, A. and Cera, G.: Evidence for a direct effect of Angiotensin II on adrenal cortex of the dog. Proc. Soc. Exptl. Biol. and Med. 109:381, 1962.

Goldblatt, H.: The renal origin of hypertension. Physiol. Rev. 27:120, 1947.

Goldblatt, H., Lynch, T., Hanzal, R. F. and Summerville, W. W.: Studies of experimental hypertension: I. Production of persistent elevation of systolic blood pressure by means of renal ischemia. J. Exptl. Med. 59:347, 1934.

Goltz, F.: Vagus and herz. Virchow's Arch. 26:1, 1863.

Goltz, F.: Ueber den tonus der Gefässe und seine bedeutung fur die blutbewegung. Virchow's Arch. 29:394, 1864.

Gross, F., Schaechtelin, G., Brunner, H. and Peters, G.: The role of the renin-angiotensin system in blood pressure regulation and kidney function. Canad. Med. Assoc. J. 90:258, 1964.

Haddy, F. J., Molnar, J. I., Borden, C. W. and Texter, E. C. Jr.: Comparison of direct effects of angiotensin and other vasoactive agents on small and large blood vessels in several vascular beds. Circulation 25:239, 1962.

Hamilton, A. S. and Collins, D. A.: Role of kidney in the maintenance of arterial blood pressure in hemorrhage. Am. J. Med. Sc. 202:914, 1941.

Hamilton, A. S. and Collins, D. A.: The homeostatic role of a renal humoral mechanism in hemorrhage and shock. Am. J. Physiol. 136:275, 1942.

Hartroft, P., Sutherland, L. E. and Hartroft, W. S.: Juxtaglomerular cells as the source of renin, further studies with the fluorescent antibody technique and the effect of passive transfer of anti-renin. Canad. Med. Assoc. J. 90:163, 1964.

Hering, H. E.: Originalien. Der karotisdruckversuch. Munch. Med. Wschr. 70:1287, 1923.

Hering, H. E.: Der sinus caroticus an der ursprungsstelle der carotis interna als ausgangsort eines hemmenden herzreflexes und eines depressorischen gefässreflexes. Munch. Med. Wschr. 71:701, 1924.

Hertting, G., Axelrod, J., and Whitby, L. G.: Effect of drugs on the uptake and metabolism of H^3-norepinephrine. J. Pharmacol. 134:146, 1961.

Heymans, C., Bouckaert, J. J., and Dautrebands, L.: Sinus carotidien et reflexes venometeurs mesenteriques. C. R. Soc. Biol. Paris 105:217 and 881, 1930.

Heymans, C., Bouckaert, J. J. and Dautrebande, L.: Sur La régulation reflexe de la circulation par les nerfs vasosensibles du sinus carotidien. Arch. int. Pharmacodyn. 40:292, 1931.

Heymans, C. and Neil, E.: Reflexogenic Areas of the Cardiovascular System. Little, Borwn and Co., Boston, Mass., 1958.

Huidobro, F. and Braun-Menendez, E.: The secretion of renin by the intact kidney. Am. J. Physiol. 137:47, 1942.

Khairallah, P. A. and Page, I. H.: Mechanism of action of angiotensin and bradykinin on smooth muscle in situ. Am. J. Physiol. 200:51, 1961.

Kirpekar, S. M. and Cervoni, P.: Effect of cocaine, phenoxybenzamine and phentolamine on the catecholamine output from spleen and adrenal medulla. J. Pharmacol. 142:59, 1963.

Koester, G. and Tschermak, A.: Ueber ursprungung und endigung des nervus depressor und nervus laryngeus superier beim kaninchen. Arch. Anat. U. Entwick (Suppl.) 255, 1902.

Kohlstaedt, K. G., Helmer, O. M., and Page, I. H.: Activation of renin by blood colloids. Proc. Soc. Exptl. Biol. and Med. 39:214, 1938.

Kohlstaedt, K. G. and Page, I. H.: Liberation of renin by perfusion of kidneys following reduction in pulse pressure. J. Exptl. Med. 72:201, 1940.

Kuhn, C., Hartroft, P. M. and Pitcock, J. A.: Effects of renal extracts on juxtaglomerular cells, renin content, and zona glomerulosa. Federation Proc. 20:404, 1961.

Laragh, J. H.: Interrelationships between angiotensin, norepinephrine, epinephrine, aldosterone secretion, and electrolyte metabolism in man. Circulation 25:203, 1962.

Lentz, K. E., Skeggs, L. T., Woods, K. R., Kahn, J. R. and Shumway, N. P.: The amino acid composition of hypertensin II and its biochemical relation to hypertensin I. J. Exptl. Med. 104:183, 1956.

Lewis, D. H. and Mellander, S.: Competitive effects of sympathetic control and tissue metabolites on resistance and capacitance vessels and capillary filtration in skeletal muscle. Acta. Physiol. scand. 56:162, 1962.

Lundgren, O., Lundwall, J. and Mellander, S.: Range of sympathetic discharge and reflex vascular adjustments in skeletal muscle during hemorrhagic hypotension. Acta physiol. scand. 62:380, 1964.

Mandel, M. J. and Sapirstein, L. A.: Effect of angiotensin infusion on regional blood flow and regional vascular resistance in the rat. Circulation Res. 10:807, 1962.

Mason, D. T. and Braunwald, E.: Effects of guanethidine, reserpine, and methyldopa on reflex venous and arterial constriction in man. J. Clin. Invest. 43:1449, 1964.

McCubbin, J. W. and Page, I. H.: Neurogenic component of chronic renal hypertension. Science 139:210, 1963.

McDowall, R. J. S.: The nervous control of the blood vessels. Physiol. Rev. 15:98, 1935.

McDowall, R. J. S.: The Control of the Circulation of the Blood. London:Dawson, 1956.

Mellander, S.: Comparative studies on the adrenergic neurohormonal control of resistance and capacitance blood vessels in the cat. Acta physiol. scand. 50: suppl. 176, 1960.

Mellander, S. and Lewis, D. H.: Effect of hemorrhagic shock on the reactivity of resistance and capacitance vessels and on capillary filtration transfer in cat skeletal muscle. Circ. Res. 8:105, 1963.

Mikasa, A. and Masson, G. M. C.: Effects of renal pressor system during hemorrhagic hypotension. Proc. Soc. Exptl. Biol. Med. 106:315, 1961.

Mulrow, P. J. and Ganong, W. F.: Role of the kidney and the renin-angiotensin system in the response of aldosterone secretion to hemorrhage. Circulation 25:213, 1962.

Oberg, B.: Effects of cardiovascular reflexes on net capillary fluid transfer. Acta Physiol. scand. 62: suppl. 229, 1964.

Page, I. H. and Bumpus, F. M.: A new hormone, angiotensin. Clin. Pharmacol. and Ther. 3:758, 1962.

Paton, W. D. M.: In: Adrenergic Mechanisms. Little, Brown and Co., Boston, Mass., 1960.

Pitcock, J. A., Hartroft, P. M. and Newmark, L. N.: Increased renal pressor activity (renin) in sodium-deficient rats and correlation with juxtaglomerular cell granulation. Proc. Soc. Exptl. Biol. Med. 100:868, 1959.

Plentl, A. A. and Page, I. H.: The enzymatic nature of angiotensin formation from renin and renin-activator. J. Biol. Chem. 147:135, 1943.

Plentl, A. A., Page, I. H., Davis, W. W.: The nature of renin activator. J. Biol. Chem. 147:143, 1943.

Reynolds, D. G. and Imig, C. J.: Segmental vascular reactivity subsequent to sympathectomy. Am. J. Physiol. 204:1145, 1963.

Robertson, P. A. and Rubin, D.: Stimulation of intestinal nervous elements by angiotensin. Brit. J. Pharmacol. 19:5, 1962.

Rose, J. C., Kot, P. A., Cohn, J. N., Freis, E. D. and Eckert, G. E.: Comparison of effects of angiotensin and norepinephrine on pulmonary circulation, systemic arteries and veins, and systemic vascular capacity in the dog. Circulation 25:247, 1962.

Sapirstein, L. A., Ogden, E. and Southard, F. D. Jr.: Renin-like substances in blood after hemorrhage. Proc. Soc. Exptl. Biol. Med. 48:505, 1941.

Sapirstein, L. A., Southard, F. D. Jr. and Ogden, E.: Restoration of blood pressure by renin activator after hemorrhage. Proc. Soc. Exptl. Biol. and Med. 50:320, 1942.

Scornik, O. A. and Paladini, A. C.: Angiotensin blood levels in hemorrhagic hypotension and other related conditions. Am. J. Physiol. 206:553, 1964.

Shadle, O. W., Zukof, M., and Diana, J.: Translocation of blood from the isolated dog's hindlimb during levarterenol infusion and sciatic nerve stimulation. Circ. Res. 6:326, 1958.

Siciliano, __: Les effets de la compression des carotides sur la pression, sur le colur et sur la respiration. Arch. ilat. Biol. 33:338, 1900.

Skeggs, L. T., Lentz, K. E., Shumway, N. P. and Woods, K. R.: Amino acid sequence of hypertensin II. J. Exptl. Med. 104:193, 1956.

Skeggs, L. T. Jr., Marsh, W. H., Kahn, J. R. and Shumway, N. P.: The existence of two forms of hypertensin. J. Exptl. Med. 92:275, 1954.

Skinner, S. L., McCubbin, J. W. and Page, I. H.: Renal baroreceptor control of renin secretion. Science 141:814, 1963.

Skinner, S. L., McCubbin, J. W. and Page, I. H.: Control of renin secretion. Circulation Res. 15:64, 1964.

Snedecor, G. W.: 1956, Statistical Methods, 5th Edition, Iowa State College Press, Ames.

Sparks, H. V.: Effects of angiotensin and desoxycorticosterol on isolated arteries and veins. U. of M. Med. Bull. 29:243, 1963.

Stone, C. A., Stavorski, J. M., Ludden, C. T., Wenger, H. C., Ross, C. A., Totaro, J. A. and Porter, C. C.: Comparison of some pharmacologic effects of certain 6-substituted dopamine derivatives with reserpine, guanethidine and metaraminol. J. Pharmacol. 142:147, 1963.

Taquini, A. C., Blaquier, P. and Taquini, A. C. Jr.: On the production and role of renin. Canad. Med. Assoc. J. 90:210, 1964.

Tigerstedt, R. and Bergman, P. C.: Niere und Kreislauf. Skand. Arch. f. Physiol. 8:223, 1898.

Tobian, L.: Relationship of juxtaglomerular apparatus to renin and angiotensin. Circulation 25:189, 1962.

Tobian, L., Janecek, J. and Tomboulian, A.: Correlation between granulation of juxtaglomerular cells and extractable renin in rats with experimental hypertension. Proc. Soc. Exptl. Biol. Med. 100:94, 1959.

Vander, A. J. and Miller, R.: Control of renin secretion in the anesthetized dog. Amer. J. Physiol. 207:537, 1964.

Whitby, L. G., Hertting, G., and Axelrod, J.: Effect of cocaine on the disposition of noradrenaline labelled with tritium. Nature Lond. 187:604, 1960.

Whitby, L. G., Axelrod, J. and Weil-Malherbe, H.: The fate of H^3-norepinephrine in animals. J. Pharmacol. 132:193, 1961.

Zimmerman, B. G.: Effect of acute sympathectomy on responses to angiotensin and norepinephrine. Circulation Res. 11:780, 1962.

Zimmerman, B. G., Gomez, J. and Stitzel, R. E.: Effect of angiotensin on vascular responses to sympathetic nerve stimulation. Pharmacologist 6:175, 1964.